Hippocampal Long-Term Potentiation: an Electrophysiological Correlate of Spatial Learning in the Rat

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Declaration

In accordance with postgraduate degree Regulation 3.4.7 of the University of Edinburgh, I declare that the work described in this document is my own, except where otherwise indicated, and that this dissertation was composed by myself.

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Abstract

Many current theories of memory formation postulate that naturally occurring changes in synaptic strength (synaptic plasticity) mediate learning. One way to study synaptic plasticity is through experimental induction of changes in synaptic strength (long-term potentiation, LTP). The relationship between LTP and spatial learning in the rat was investigated here in a two-part study. Part 1 tested the hypothesis that artificially driving hippocampal synapses to their maximum strength would preclude any further naturally occurring changes and so disrupt the subsequent spatial learning function of the hippocampus. The experiments were conducted in a watermaze using a spatial learning task which is known to require an intact hippocampus. A failure of rats with such a "saturation" of synaptic strengths to learn a spatial task would (a) support the hypothesis that the hippocampus is involved in spatial learning, (b) support the hypothesis that naturally occurring synaptic plasticity is needed for such a spatial learning function, (c) replicate the findings of two previous studies in which just such a saturation-related learning impairment had been observed and (d) provide a basis for further studies intended to probe the contribution of individual hippocampal pathways to spatial learning. However, no learning impairment was seen, even when the conditions of one of the previous studies were repeated exactly. Possible reasons for this are discussed. It was observed, however, that in individual animals the cumulative level of LTP correlated highly with performance on the spatial task, supporting the idea that synaptic plasticity and learning may nevertheless be closely related.

Part 2 investigated the above LTP-learning correlation in more detail. First, the order of training and LTP induction was reversed to see whether induction of LTP may have caused the corresponding distribution of spatial learning seen previously. Although poor learning rats again showed less LTP the correlation was much lower than in the previous experiments, both for the group trained first and the group trained afterwards. Possible reasons were explored in the final experiment. Animals were spatially trained prior to LTP induction and classically conditioned afterwards, and LTP was measured across a wide range of stimulus intensities. When measured at a high test stimulus intensity LTP correlated positively with spatial learning, as before, but this relationship reversed with lower current strengths. A similar though less marked relationship existed for the discrimination task, suggesting an interaction with spatial learning. The failure of other investigators and the previous experiment to see such an LTP-learning correlation may be because LTP is usually measured at moderate stimulus intensities, in the region of the crossover of this relationship. The most likely and interesting reason for such a crossover

is that paradoxically, poor-learning animals have more plastic synapses but that the effects of non-linear summation of population post-synaptic potentials mask this increase unless the test stimulus intensities used are very small.

The two main results of this thesis therefore are that (1) contrary to previously reported findings, induction of LTP in the perforant path does not appear to impair spatial learning, and (2) there is a relationship between underlying synaptic plasticity and learning ability. It appears that this relationship may lie in the opposite direction to that predicted by computational theories of learning: namely, animals showing poor learning ability can demonstrate increased rather than decreased plasticity. These results provide clear support for a link between hippocampal synaptic plasticity and spatial learning but suggest that the relationship may be more complex than is generally supposed.

Acknowledgements

As with any Ph.D. thesis, this work was only able to come about because of the combined efforts of several people. First and foremost I would like to thank my supervisor, Richard Morris, whose lucid writing on the subject of synaptic plasticity and learning was largely responsible for my initially becoming interested in the field. His watchful but non-interventional approach to supervision allowed me to wander close to experimental pitfalls without actually falling into any of them, something which must have cost him a great deal of anxiety (which he kindly concealed). As a result I learned much from him about experimental design, and how to ask interesting questions and to fit the answers into the broader picture.

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Abbreviations

5-HT	5-hydroxytryptamine, serotonin
AMPA	amino-3-hydroxy-5-methyl-isoxazole-4-propionate
A-P	antero-posterior
AP5	amino-5-phosphonopentanoate
CA(1,2,3,4)	cornu ammonis subfields
CAMK II	Calcium-calmodulin-dependent protein kinase II
CPP-ene	3-[(±)-2-carboxypiperazin-4-yl]-1-propenyl-1-phosphonic acid
CS+, CS-	conditioned stimulus, rewarded or non-rewarded
DA	dopamine
DG	dentate gyrus subfield
DHC	dorsal hippocampus
EC	entorhinal cortex
ECS	electroconvulsive shock
EEG	electroencephalograph
EPSP	excitatory postsynaptic potential
EPSC	excitatory postsynaptic current
E-S potentiation	EPSP-spike potentiation
GABA	γ-aminobutyric acid
HF	high frequency
i.m.	intra-muscular
IO curve	input-output curve
i.p.	intra-peritoneal
IPSP	inhibitory postsynaptic potential
LF	low frequency
LTD	long-term depression
LTP	long-term potentiation
MK-801	(+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine maleate
M-L	medio-lateral
NA	noradrenaline
NMDA	*N*-methyl-D-aspartate
STP	short-term potentiation
US	unconditioned stimulus
VHC	ventral hippocampus

Chapter 1 – General Introduction

1.1 Overview

One of the current major goals of neurobiology is to understand how the brain assimilates information about objects and events in the outside world and stores it in the form of memories, to be retrieved and used at a later time. The phenomenology of learning and memory in humans and animals suggests that the process is very unlike the artificial forms of information storage produced by technological developments such as writing and, more recently, computers. Biological memory formation is flexible and adaptive, and memory retrieval is extremely rapid. An understanding of the mechanisms of this type of information storage would shed light on a scientific puzzle which is of great interest. Furthermore, elucidation of the biology of memory formation may produce therapies for presently intractable clinical conditions such as the amnesias and the psychoses, as well as being of considerable technical use.

There is at present no good general theory of how cognitive processes such as learning and memory could come about from the interaction of the large numbers of small units (*i.e.* neurons) which make up the brain. However, although a complete model is still lacking, initial steps have been taken by the collaboration of experimental neurobiology with the theoretical field of connectionism. The latter discipline dates back at least as far as the neuroanatomist Ramón y Cajal, who suggested early this century that information could be processed by changes in the synaptic connections between neurons (Ramón y Cajal, 1911). This idea was later formalised by Hebb, who postulated that information could be stored by increasing the strengths of the connections between groups of simultaneously active nerve cells in order to form associations (Hebb, 1949), a postulate which contributed to the development of the first computational models of learning. At the present time, neurobiology and connectionism are interacting in order to develop an understanding not only of the implementational details of the workings of modern day organisms, but of how high level processes such as cognition could, in principle, be produced independent of their substrate.

The experiments to be described in this thesis were designed against a backdrop of connectionist theory which postulates that information storage by neurons is mediated by a Hebbian (or Hebb-like) modification of synaptic strength. It was not until some years after Hebb proposed his synaptic modification rule that experimenters began looking for physiological evidence of variability in synaptic strength in real neurons. Following an

initial abstract (Lømo, 1966) a group of researchers published, in 1973, the first detailed reports of artificially induced modification of synaptic strength. These experiments were conducted in the hippocampus, a prominent structure in the temporal lobe which appears to play a critical role in the formation of some types of memory. It was found that if the main afferent pathway into the hippocampus was stimulated with strong high-frequency electrical pulses, then the synapses of those fibres onto postsynaptic cells became measurably stronger and stayed so for many weeks (Bliss and Lømo, 1973, Bliss and Gardner-Medwin, 1973). After some debate (Bliss and Lynch, 1988, p.3) this phenomenon came to be termed long-term potentiation (LTP) and subsequent studies showed that LTP could only be induced if the input fibres were stimulated while the postsynaptic cells were already active (that is, sufficiently depolarised). The parallel with the Hebb rule was immediately apparent.

The finding that synaptic plasticity (as presently measured by ease of LTP induction) is such a conspicuous feature of a memory structure like the hippocampus provides important support for the connectionist hypothesis that variation in synaptic strength mediates learning. However, although it has been known for more than two decades that Hebb-type synaptic modifiability exists in the mammalian brain, it has been somewhat difficult to demonstrate either that this plasticity arises under natural conditions in a normally behaving organism, or that such naturally-occurring modifications are mediating learning rather than performing some other non-specific modulatory function. Such a demonstration is important. If synaptic modifiability should indeed turn out to mediate the learning function of the hippocampus then connectionist theory would have achieved a significant victory, and the foundations could be laid for further experiments aimed at determining exactly *what* information is being stored and what the representation looks like. If synaptic modifiability should turn out not to be mediating learning then connectionists must re-design their models and biologists must look for some other memory mechanism. The aim of the present work therefore was to join the current search for links between synaptic plasticity and learning, motivated by the belief that the finding of such links would represent an important step forward in the understanding of biological memory formation.

1.2 Background

The hippocampus

Since the discovery of LTP, much experimental work on the possible role of synaptic plasticity in learning and memory has continued to focus on the hippocampus. There are several reasons for this. First, although its exact function is still debated, the hippocampus appears to play an important part in forming some types of memory. In the rat, a wealth of

psychological studies, some of which are described in a later section, indicate that the memory function subserved by the hippocampus may be predominantly related to the learning of spatial relationships within the environment. The computational problems which need to be solved in order for an organism to be able to represent the environment and navigate around it appear intuitively more tractable than more high-level processes such as language generation, and for this reason spatial learning is an attractive paradigm with which to study elementary cognitive processes.

Second, the hippocampus is a rather beautiful structure, precisely organised with a complex and very patterned geometry. The regularities of its design suggest that some well-defined computational process is being undertaken, and one which could be amenable to theoretical modelling if enough were to be discovered about the details of its inputs and connectivity. If a theoretical description of the computation being undertaken by the hippocampus can be combined with a sufficiently detailed knowledge of its anatomy, then it may be possible to match structure with function: in other words, to discover how the hippocampus works. The principles governing its function may then also generalise to other brain structures.

Finally, although LTP has been discovered in other cortical structures, it is far more readily elicited in the hippocampus. This may be because the regularity of its design makes it possible for an experimenter to persuade large numbers of cells to act synchronously, facilitating production of the conditions under which LTP occurs. It may also be an indication that the specialised memory function of the hippocampus is mediated by an LTP-like process: a hypothesis which, if subsequently validated, would greatly facilitate the formation of a computational model of hippocampal function.

LTP and the Hebb rule

The rule proposed by Hebb to govern the conditions under which changes in synaptic strength might occur runs as follows:

> *When an axon of cell A is near enough to excite a cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficacy, as one of the cells firing B, is increased. (p.62)*

It may be seen that the Hebb rule allows for the association of neural events. If cell A is connected to cell B and they are both active simultaneously, for whatever reason (other inputs may also be activating either or both cells) and the connection between them is therefore strengthened, in accordance with the Hebb rule, then the future occurrence of activity in cell A is more likely to produce activity in cell B as well. Such association of

events forms the basis of many learning theories (*e.g.* Pavlov, 1928, Hull, 1943, Rescorla and Wagner, 1972).

When hippocampal LTP was discovered, it was immediately apparent that it provided a potential physiological mechanism for implementing the Hebb rule. At the present time, considerable advances have been made in the understanding of the physiological mechanisms by which LTP induction is brought about. However, the evidence concerning the functional role that synaptic plasticity might play in the life of a normal animal is still sparse. The purpose of the work described in this thesis was to investigate the possible relationship between the experimental phenomenon of LTP and the natural phenomenon of memory formation.

Before describing the experiments to be presented in the next five chapters, the current literature concerning evidence of links between synaptic plasticity and learning will be reviewed. First, however, it is necessary to present some of the basic anatomy and physiology of the hippocampus, in order to provide a framework for subsequent discussion. Because the present work lies on the boundary between physiology and psychology, an attempt has been made to present background information in a form accessible to specialists of either discipline.

1.3 Anatomy and physiology of the hippocampus

1.3.1 Anatomy

Much of our current knowledge of hippocampal anatomy and circuitry is due to the early studies of Ramón y Cajal (1911), Lorente de Nó (1933, 1934), Blackstad (1956, 1958) and Hjorth-Simonsen (1973). The anatomical description which follows pertains to the rat, and much of it has been summarised from the review by Amaral and Witter (1989). These "implementational" details are important because they determine the forms of processing which are in principle possible by the brain and therefore constrain theoretical models, as well as clarifying the interpretation of experimental results.

Major subregions

The shape of the hippocampus and its relationship to the rest of the brain is shown in Fig. 1.1. In the rat, the hippocampus encircles the thalamus and is composed of the left and right hippocampi, which are joined at the midline anteriorly by the hippocampal commissure but separated by several mm more posteriorly. The anterior ends lie near the septal nuclei and the posterior ends lie near the amygdala and entorhinal cortex. Moving posteriorly (and temporally), each hippocampus curves latero-ventrally around the thalamus so that a cross-section taken perpendicular to its surface becomes (with respect

to the rest of the brain) first coronal and then progressively more horizontal. Because of the inclined orientation of the hippocampus, its septal pole is often alternatively referred to as the dorsal end and the temporal pole as the ventral end. Because of the curvature, to simplify description it is customary to refer the orientation of cross sections to a curved long axis running down through its centre as if it the hippocampus had been unrolled and then sliced, as is done for *in vitro* experiments. The term "septo-temporal" is often used to refer to this longitudinal axis.

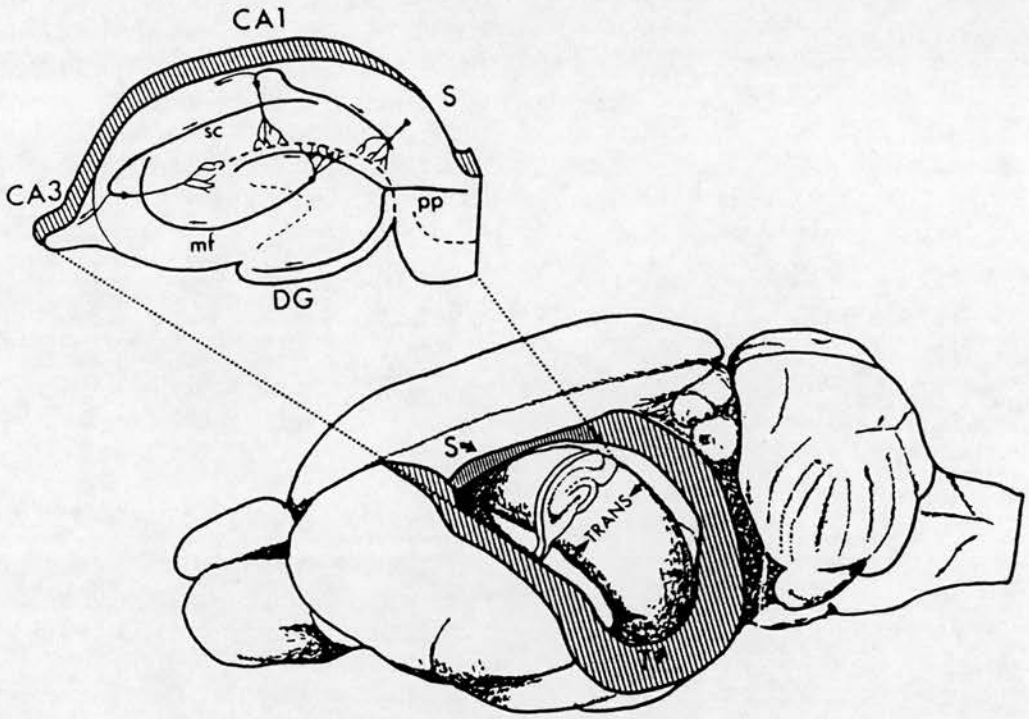

Figure 1.1 (Taken from Andersen *et al.*, 1971b) Illustration of the rat brain with part of the left-hemisphere cortex removed to reveal the underlying left hippocampus. S marks the septal pole and T the temporal pole. The expanded cross-section in the upper region of the diagram shows the 2 interlocking "C"-shaped cell fields which comprise the dentate gyrus (DG) and cornu ammonis (CA3 and CA1).

After unrolling, the hippocampus is seen to consist of two interlocking sheets of cells folded in half lengthwise (Fig. 1.2). The boundary between the uppermost layer of cells and the next deepest layer is made by the obliterated hippocampal fissure. The two interlocking layers are principally composed of morphologically different cell types and are known as the dentate gyrus (DG), mainly consisting of granule cells, and the hippocampus proper or cornu ammonis (usually abbreviated to CA) which mainly consists of pyramidal cells.

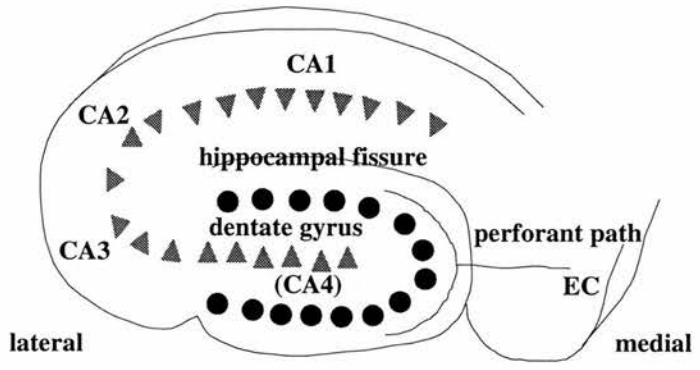

Figure 1.2 Schematic cross-section of the hippocampus made perpendicular to its long axis, showing the major subfields: dentate gyrus, CA3, CA2 and CA1. CA4 is generally regarded as a subset of the CA3 region. EC = entorhinal cortex. The major cell type in the dentate gyrus is the granule cell (black circles) and in the hippocampus proper the pyramidal cell (grey triangles). Also shown is the termination of a single perforant path afferent from the entorhinal cortex onto dentate granule cells.

The dentate gyrus lies immediately below the hippocampal fissure and curves around so that its two arms encompass the pyramidal cells of the lower arm of the hippocampus proper. For this reason the upper arm of the dentate gyrus is sometimes known as the suprapyramidal blade and the lower arm the infrapyramidal blade. The region between the two blades near the fold is called the hilus. The subregions of the cornu ammonis are numbered from 1 to 4, starting from its most superficial region and moving around to the arm lying between the blades of the dentate gyrus. Of these subregions CA1 and CA3 are the most separable, on histological and physiological grounds. CA4 is usually regarded as a subset of CA3.

The other components of the hippocampal formation are the entorhinal cortex (EC) and the subicular complex, which form its major input and target respectively. The EC is composed of two major subdivisions, medial and lateral. It receives highly processed multimodal sensory information from sensory cortex and projects to the dentate gyrus via a fibre bundle known as the perforant path. In the rat, the medial entorhinal cortex gives rise to the medial perforant path and the lateral entorhinal cortex to the lateral perforant path (Hjorth-Simonsen and Jeune, 1972, McNaughton and Barnes, 1977), both of which project strongly to the ipsilateral dentate gyrus and weakly to the contralateral DG (Steward, 1976). Perforant path fibres leaving the entorhinal cortex form a compact bundle known as the angular bundle, before spreading out and distributing themselves among various regions, making *en passage* synaptic contacts with their target cells. Fibres crossing the hippocampal fissure to reach the dentate gyrus bifurcate and supply a branch to both the supra- and infrapyramidal blades. The dendritic zone of the dentate gyrus which receives these inputs is called the molecular layer. Afferents from the medial EC arriving via the medial perforant path terminate on the middle third of the dendrites and those coming from the lateral EC via the lateral perforant path terminate on the distal third (Steward, 1976).

The subicular complex receives input from the CA1 output of the hippocampus, as well as projecting to and receiving projections from the entorhinal cortex. Its three major subdivisions are the subiculum, presubiculum and parasubiculum. The only subcortical outputs of the hippocampus proper are a bilateral and unilateral projection to the lateral septum from CA3 and CA1 respectively (Jarrard, 1983, Swanson and Cowan, 1977).

Subcortical inputs

As well as a large input from the perforant path, the hippocampus receives a sparse but important input from subcortical structures such as the thalamus and hypothalamus, brainstem, septum and amygdala. These inputs carry information about arousal, emotions and autonomic functions, and influence the hippocampus via modulatory substances such as serotonin (5-hydroxytryptamine, 5-HT), noradrenaline (NA), dopamine (DA) and histamine. The septum also modulates hippocampal activity via acetylcholine and γ-aminobutyric acid (GABA; Freund and Antal, 1988) to produce the prominent sinusoidal hippocampal electroencephalographic (EEG) activity known as "theta" rhythm (Green and Arduini, 1954).

Circuitry

The circuitry of the hippocampal formation is complex and not yet fully determined. Its outstanding feature is a predominantly one-way flow of excitation from the entorhinal cortex to the dentate gyrus, through CA3 to CA1 and thence to the subicular complex and back to the EC (Fig. 1.3A). This sequence of connections forms the classical "trisynaptic circuit". The orderly connectivity of the trisynaptic circuit is sufficiently compelling to have led early investigators to postulate that information flow through the hippocampus takes place primarily in this direction, orthogonal to the long axis, and that there is very little communication longitudinally. The so-called "lamellar hypothesis" proposed by Andersen and colleagues (1971b), and supported by their electrophysiological findings, proposed that the hippocampus was divided into functional units consisting of slices orientated transverse to the long axis. Incoming information would be routed through the trisynaptic circuit and each slice would carry out its processing more-or-less autonomously. More recent anatomical studies have suggested that longitudinal flow of information is actually much greater than previously realised (Amaral and Witter, 1989) and that the view of the hippocampus as a series of parallel functionally independent units should probably be revised in favour of a more integrated model. In addition, although recent findings still support the unidirectionality of information flow, there appear to be a number of "short-cuts" such that the trisynaptic circuit is only one of several possible ways in which information could pass from the EC to the subiculum (Fig. 1.3B).

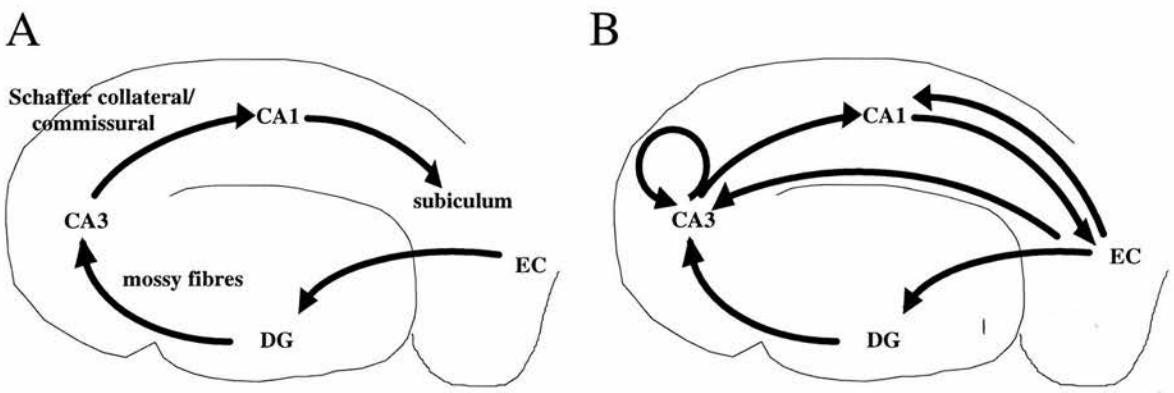

Figure 1.3 (A) Unilateral flow of excitation through the "trisynaptic circuit" from the entorhinal cortex (EC) through the perforant path fibre bundle to the dentate gyrus (DG), through the mossy fibre system to CA3 and through the Schaffer collateral/commissural pathway to CA1. The output from CA1 projects to the subicular complex (not shown), part of which projects back to the EC. (B) A more detailed diagram of the connections showing local recurrent circuitry in CA3, and direct "short-cut" connections from EC to CA3 and CA1, as well as feedback from CA1 to the EC.

Neuronal subtypes

There are several types of neuron in the hippocampus, of which the three major classes are the granule cells, pyramidal cells and basket cells. Granule cells are the principal cells of the dentate gyrus and pyramidal cells, which resemble the pyramidal cells found in all other parts of the cortex, are the principal cells of the hippocampus proper. Both cell types are excitatory, probably using glutamate as a transmitter. Basket cells are interneurons found throughout all regions of the hippocampus and are GABA-ergic (Sloviter and Nilaver, 1987).

Granule cells possess a highly branched dendritic system (dendritic arbour). The dendrites pass from the cell bodies up through the molecular layer to the cortical surface, and are studded with small protuberances known as spines. The dendrites receive their synaptic contacts mainly from perforant path afferents, although there is also a dense local (though predominantly contralateral) input from a population of cells known as "mossy cells" which are found in the hilus (Amaral, 1978). It has been estimated that approximately 400 afferent fibres must be active in order to bring a granule cell to firing threshold (McNaughton *et al.,* 1981). The granule cell axons, known as mossy fibres, make excitatory contacts with CA3 cells.

Pyramidal cells are oriented with the apices of their pyramids facing towards the hippocampal fissure. Dendrites arise from the base and the apex of the pyramids and receive afferent inputs from other parts of the hippocampus via commissural and Schaffer collateral fibres, and from the cortex via the perforant path.

Basket cells are large neurons located near or within the granule cell and pyramidal cell layers. Their dendrites are aspinous and receive inputs from the same afferents as the

principal cells as well as local axon collateral inputs. Their output is a GABA-mediated inhibition which projects locally, allowing them to mediate both feedforward and feedback inhibition (Amaral, 1978).

Architecture of the dentate gyrus

The unusual architecture of the dentate gyrus makes it possible to obtain stable recordings of synchronous population behaviour from its cells over considerable periods of time (days to weeks). For this reason long-term studies of hippocampal physiology, such as in the present work, often focus on this system.

Dentate granule cells are densely packed in a single curved layer in the dentate gyrus (Fig. 1.4). Because of the bifurcation of perforant path fibres, stimulation of the angular bundle produces nearly synchronous activation of granule cells (Lømo, 1971).

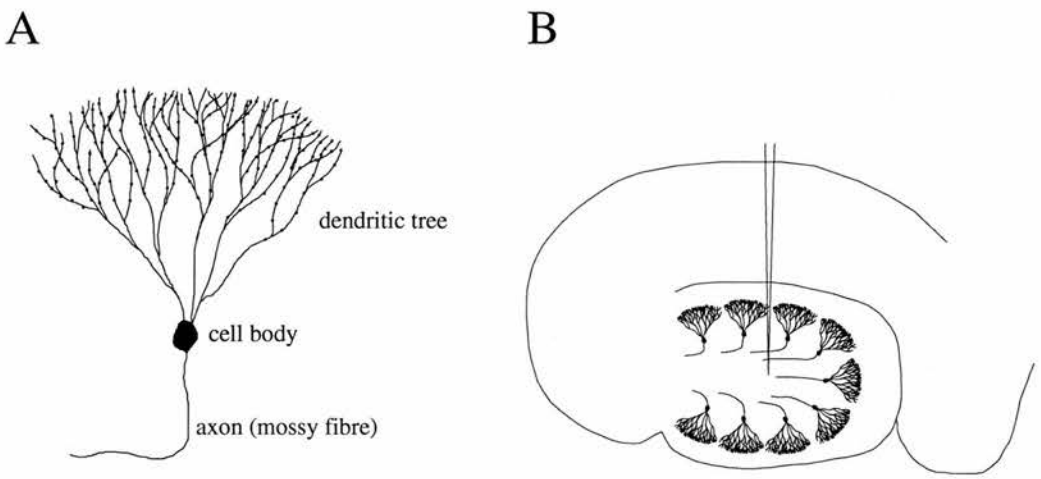

Figure 1.4 (A) Morphology of a dentate granule cell, showing the extensive dendritic arbour with spines, and mossy fibre axon. (B) A recording electrode situated in the hilus of the dentate records evoked potentials from an approximately spherical surround of hilar cells.

The curvature of the dentate gyrus means that it effectively behaves like a closed sphere of cells. In this type of configuration, a recording electrode situated between the two layers of cells will see approximately the same evoked membrane potential whether it is near the cell bodies or in the centre of the hilus, meaning that movements of a microelectrode throughout this region will not substantially alter the recorded potential (Lømo, 1971a; McNaughton, 1980; see below). This allows for great stability of chronic preparations.

1.3.2 Electrophysiology

Normal evoked responses to perforant path stimulation

All of the work described in this thesis was conducted in the perforant path and dentate gyrus, so a review of hippocampal physiology will be provided mainly with reference to

this system. A comprehensive analysis of the components of population granule cell responses to perforant path stimulation may be found in Lømo (1971a). The medial and lateral components of the perforant path between them project to the entire septo-temporal extent of the dentate gyrus, making excitatory synaptic connections in the molecular layer. The fibres also make excitatory contact with basket cells connecting to dentate granule cells, producing a feed-forward inhibitory influence on the granule cell response. In addition, the granule cells themselves contact basket cells, producing feed-back inhibition. A stimulating electrode placed in the angular bundle of the perforant path will therefore exert a monosynaptic excitatory effect and a di- and tri-synaptic inhibitory effect on the target granule cells.

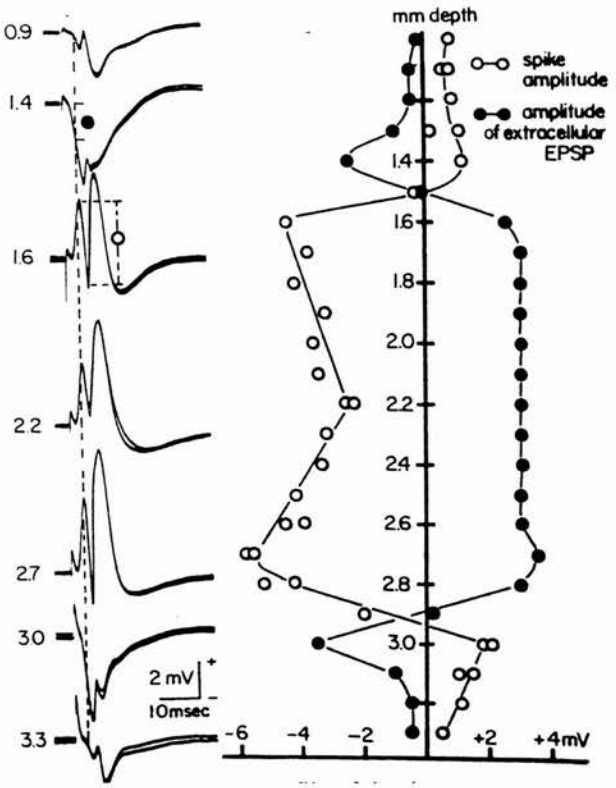

Figure 1.5 (Taken from Lømo, 1971a) Depth profile of the dentate response to a perforant path stimulus. During its descent the recording electrode first passes through the molecular layer of the upper blade of the dentate gyrus, then the cell body layer, hilus, cell body layer of the lower blade and lower molecular layer. When the electrode is in the dendritic zones the field potential is negative. In the cell body layer and hilus it reverses polarity and becomes positive. There is relatively little change in the size of the response as the electrode traverses the hilus.

Lømo (1971a) investigated the shape of the population granule cell response (the field potential) at varying depths below brain surface to a stimulating pulse applied via an

electrode in the perforant path (Fig. 1.5). As the recording electrode passes through the dendritic (molecular) layer, where perforant path fibres terminate, a negative wave is observed, reflecting the influx of positive ions into the dendrites (current sink; Andersen *et al.,* 1966). As the cell body layer (the current source) is approached, the potential reverses and becomes positive. The positive wave is the excitatory post-synaptic potential (EPSP), upon which is superimposed a sharp negative potential, the population spike, which results from the synchronous firing of many granule cells (Andersen *et al.,* 1971a). In the region between the upper and lower blades of the dentate gyrus, as discussed above, the evoked response changes relatively little because of the spherical configuration of the cells. In chronic implantations, therefore, the recording electrode is typically positioned in the hilus.

Long-term potentiation

Bliss and colleagues (Bliss and Lømo, 1973, Bliss and Gardner-Medwin, 1973) discovered that if the single test pulses described above are replaced by a "train" of high-frequency (from 100-400 Hz) pulses, the subsequent size of the response evoked by the test pulses increases and stays increased for some time, a phenomenon which they named "long-term potentiation" (LTP). In chronic animals LTP may last for many weeks (Bliss and Gardner-Medwin, 1973, Barnes, 1979, Racine *et al.,* 1983, Jeffery *et al.,* 1990). LTP has attracted a great deal of attention because some of its properties are suggestive of those which should be possessed, in theory, by a memory mechanism (*e.g.* Hebb, 1949). In addition to its long-lasting nature, the properties which make it computationally interesting are associativity, cooperativity and input-specificity (Bliss and Collingridge, 1993). Associativity means that LTP induction in a given synapse may be regulated by other convergent inputs terminating on spatially distant regions of the postsynaptic cells, so that the relevant synapse may not even need to have been tetanised in order to become potentiated (Wigström *et al.,* 1986). Cooperativity is a closely related concept, and refers to the fact that a greater stimulus intensity during tetanisation will produce greater LTP because the larger number of stimulated fibres interact to produce a mutual facilitation of LTP induction (McNaughton *et al.,* 1978). Specificity refers to the finding that changes in strength only take place on synapses that had been active (*i.e.* releasing neurotransmitter) at the time that the LTP-inducing event took place, or within a short time (up to 200 ms) on either side of it. Other synapses contacting the same postsynaptic cell will not become potentiated if they were not active at the time (Andersen *et al.,* 1977, McNaughton and Barnes, 1977, but see Bonhoeffer *et al.,* 1990). These properties all derive from an underlying induction requirement, which is that for LTP to occur the postsynaptic cell must be strongly depolarised at the same time as the presynaptic terminals are releasing neurotransmitter. It is now known that in most regions of the hippocampus (though not

all: see Harris and Cotman, 1986, Grover and Teyler, 1990 and Bramham *et al.,* 1991 for exceptions) these induction properties can largely be attributed to a specialised postsynaptic receptor, the NMDA receptor, which will be described in greater detail below. Briefly, its characteristics are such that its associated ion channel will only open when the postsynaptic membrane on which it resides is depolarised past a certain threshold, and at the same time neurotransmitter (probably L-glutamate) is released into the synaptic cleft. Both of these conditions are satisfied during the massive synchronous activation and postsynaptic depolarisation which occurs during a tetanus, but it appears that any method of achieving the two conditions will suffice to trigger LTP induction. For example, LTP will also occur if the postsynaptic cell is artificially depolarised and paired with single pulses to afferent fibres (Wigström *et al.,* 1986, Gustafsson and Wigström, 1986, Wigström and Gustafsson, 1986). Conversely, hyperpolarisation of the postsynaptic cell blocks LTP induction even when all the other usual requirements are met (Malinow and Miller, 1986).

A question which remains unanswered is whether the factors needed to trigger LTP induction ever occur naturally: that is, whether LTP is merely an artefact of a highly unusual set of conditions brought about by an experimenter, or whether it is a manifestation of a naturally occurring process which normally takes place in a much smaller subset of synapses. One of the difficulties in relating the induction conditions described above to naturalistic learning in awake, behaving animals is that under normal conditions it is highly unlikely that entorhinal or hippocampal cells would ever fire synchronously at the high frequencies previously needed to induce LTP in the laboratory. However, it is gradually becoming clear that LTP can sometimes be elicited under conditions which are more physiological: that is, following patterns of afferent activity which resemble those which have been observed to occur naturally. For example, Buzsáki *et al.* (1987) found that pairing single pulses with spontaneous bursts of cellular activity ("sharp waves", produced by blocking basket cell inhibition) could induce LTP. A subthreshold burst of pulses or a single stimulus (known as priming stimulation) followed by a short train of 2-10 pulses induces robust LTP if, and only if, the priming stimulation precedes the train by an interval of 150-200 ms (Larson *et al.,* 1986, Diamond *et al.,* 1988, Greenstein *et al.,* 1988). This suggests that the temporal patterning of stimulation and not just its strength is important for inducing LTP. This finding is interesting because the 150 ms interval corresponds to the frequency of endogenous rhythmic hippocampal activity (theta), suggesting that there may be a link between theta and naturally occurring LTP. It is now believed that the priming interval results from depression of inhibition by negative feedback onto inhibitory (GABA-B) autoreceptors (Davies *et al.,* 1991), allowing greater postsynaptic depolarisation.

Taken together, these findings suggest that although LTP is an artificial phenomenon, it depends upon cellular machinery which may plausibly be used under real-life conditions to produce a similar end result: that is, modulation of synaptic efficacy. The term "long-term potentiation" (LTP) specifically refers to the type of experimentally-induced change in synaptic strength brought about in a laboratory under artificial conditions. The term "synaptic plasticity" is commonly used synonymously with LTP, but the former term is more accurately used to mean the *capacity* for change, to be distinguished from the actual changes themselves. In addition, "LTP" is often used loosely to mean a change in synaptic strength when in reality there may be several causes of such a change, of which LTP is only one. To avoid confusion, the term "synaptic plasticity" will herein be used to mean the propensity for synapses to change their strengths in response to stimulation, irrespective of whether the necessary stimulation was actually applied. Learning-associated change in synaptic strength (if there is such a thing) will be referred to as "naturally occurring LTP", and the term "LTP" will be used to refer only to the experimentally-induced subset of synaptic plasticity which lasts longer than 30 min after administration of the stimulus used to induce it. It is worth noting that even this relatively restrictive definition may encompass a cluster of interacting phenomena (see below).

Short-term potentiation phenomena and phases of LTP

Analysis of the time course of the decay of LTP suggests that more than one process probably underlies the increase in evoked response size following high frequency stimulation. It has been known for some time that several transient alterations in evoked response size can be induced at neuromuscular junctions following high frequency electrical stimulation (Magelby and Zengel, 1976). McNaughton (1980) investigated very-short-term potentiation phenomena in the rat perforant path/dentate synapse and found a closely related pattern of response changes to high-frequency, low-intensity stimulation, which he classed according to increasing time course as facilitation, augmentation and potentiation. The time constants of these processes are approximately 100 ms, 5 s and 20-240 s respectively. Racine and Milgram (1983) also observed a longer-lasting component with a time constant of 6.5 min. In further studies involving high frequency (LTP-inducing) stimulation, Racine *et al.* (1983) found that LTP itself had at least 2 time constants, of 1-2 h and 5 days respectively, which they termed LTP1 and LTP2. The short-lasting phase of LTP immediately following tetanisation is also called short-term potentiation (STP) and may correspond to LTP1. Barnes (1979) found that LTP decayed with a time constant of 3 days in old rats and 3 weeks in young rats. Jeffery *et al.* (1990) found that an LTP-induction protocol which induced long-lasting LTP (with a time constant of about 2 weeks) in awake animals induced short-lasting LTP (time constant of less than 5 days) in pentobarbital-anaesthetised rats. They suggested that the

long-lasting form of LTP represented a third phase, which they termed LTP3. The physiological basis of the various phases of LTP has yet to be elucidated, but may involve transcriptional processes in the early stages and translational processes (perhaps including immediate-early gene activation) in the later stages.

1.3.3 Pharmacology

The main excitatory neurotransmitter in the hippocampus is L-glutamate, which acts on both pre- and postsynaptic receptors. The postsynaptic receptors are located principally on dendritic spines, and three types are currently known: the AMPA receptors (previously known as kainate/quisqualate) which mediate fast synaptic transmission through Na^+ and K^+ ion channels, the NMDA receptors which mediate a slow depolarisation (through Ca^{2+} channels) and the metabotropic receptors, which are not coupled to ion channels but which directly activate a second messenger signalling system. When a fibre is stimulated, Ca^{2+} influx into the axon terminals causes release of glutamate in the usual way. The glutamate diffuses across the synaptic cleft and binds to all three types of receptor. Its action on AMPA receptors is to cause Na^+ influx which depolarises the cell. Its action on the metabotropic receptor is not fully elucidated at present and will not be considered further here, although it may contribute to LTP induction (Bliss and Collingridge, 1993). The action on NMDA receptors is complex, and because the NMDA receptor plays a crucial role in initiating the formation of LTP (Collingridge *et al.,* 1983, Harris *et al.,* 1984, Errington *et al.,* 1987), it will be described in some detail.

The NMDA receptor

In the hippocampus, NMDA receptors are found most densely in the CA1 subfield, and also in the dentate gyrus, though sparsely in the stratum lucidum of the intervening CA3 subfield (Monaghan and Cotman, 1985). They are located on the postsynaptic density and are frequently co-localised with other types of glutamate receptor, particularly the AMPA receptors (Young and Fagg, 1991). They differ from these receptors, however, in that they have the unusual properties of (a) requiring the binding of two agonists, glutamate and glycine (Bashir *et al.,* 1990) in order to be activated (Johnson and Ascher, 1987), (b) activation which is voltage dependent (Collingridge *et al.,* 1988a) and (c) permeability of its associated ion channel to Ca^{2+} (Dingledine, 1983).

Activation of an NMDA receptor results in the opening of its associated Ca^{2+} ion channel only when the postsynaptic cell has been sufficiently depolarised by some other means. This voltage dependence arises because of the fact that, under resting voltage conditions (usually around -65 mV), the ion channel is blocked by Mg^{2+} ions (Nowak *et al.*, 1984). A single electrical pulse applied to the perforant path will therefore not activate NMDA receptors because the degree of depolarisation is not sufficient to unblock the channel

(Coan and Collingridge, 1985). When the cell becomes strongly depolarised, for example during a high frequency train of pulses, the Mg^{2+} ions vacate the channel, allowing Ca^{2+} ions to flow into the dendritic spine. The flow of ions can be measured as a long, slow depolarising potential reaching a peak at 10-70 ms and lasting more than 250 ms (Collingridge et al., 1987, 1988b), on top of which are superimposed the usual fast (1-2 ms) EPSPs triggered by other types of ion-channel-associated receptor and mediated by Na^{+} ions. This inflow of ions triggers a self-reinforcing chain of events which results in a complex array of changes in the postsynaptic cell, culminating in the induction and expression of LTP.

The dual activation requirements of the NMDA receptor endow it with properties of potential computational importance. One such property is that, in theory, the receptor may be a mechanism for detecting conjunctions of activity from two or more separate inputs onto a single set of postsynaptic cells, and, therefore, a mechanism for implementing the Hebb rule (Wigström and Gustaffson, 1985, Cotman and Monaghan, 1988). Recall that the Hebb rule for synaptic modification states that increases in synaptic strength should occur when an active input contacts an already-activated target. The NMDA receptor accomplishes this by only opening its ion channel (which will set in motion the chain of events culminating in LTP) in response to agonist binding when the cell on which it resides has been sufficiently depolarised by whatever inputs it is currently receiving.

Neuromodulation of LTP

Because the hippocampus receives diffuse ascending neuromodulatory inputs, it might be expected that these would play a role in regulating the induction of LTP. Bliss *et al.* (1983) investigated the effects on granule cell excitability and on LTP induction of depleting the forebrain of the monoamines NA and 5-HT. NA depletion reduced LTP magnitude significantly while leaving both the short-term potentiation (STP) component and the LTP decay rate unchanged. 5-HT depletion produced a more profound reduction of LTP and also shortened its time course. In addition, 5-HT depletion produced an increase in baseline granule cell excitability. Interestingly, while NA depletion did not change the baseline evoked response, it did abolish the rise in population spike size seen in the untetanised hemispheres of control animals, a rise which in the light of recent findings concerning the effects of temperature changes on evoked responses (Moser *et al.*, 1993) may be attributable to cooling of the animals during surgery (see Section 1.4.2).

GABA is also involved in the modulation of LTP induction, in a somewhat complex manner. Blockade of inhibition by picrotoxin facilitates LTP induction (Wigström and Gustafsson, 1983), due to the resulting increased postsynaptic depolarisation. During an electrical tetanus, however, the GABA-ergic inhibitory component of the evoked potential acts on presynaptic GABA-B autoreceptors to depress further release of GABA,

thus effectively fatiguing inhibition and allowing sufficient postsynaptic depolarisation to unblock the NMDA receptor (Davies *et al.,* 1991). Other factors which have been implicated in the modulation of LTP induction include opioids (Bramham *et al.,* 1988, Shors *et al.,* 1990), corticosteroids (Diamond *et al.,* 1989, Pavlides *et al.,* 1993) and growth factors (Morishita *et al.,* 1992). As will be discussed in the next section, many of the effects of these agents on LTP resemble their effects on learning, lending support to the hypothesis that LTP and learning share a common mechanism.

To summarise, the hippocampus appears to possess cellular machinery which could, in principle, fulfil the role of a conjunction detector in the manner predicted by computational theories of learning such as Hebb's postulate. Furthermore, it is possible in a laboratory setting to use this machinery, following such a conjunction (coincidence of pre- and postsynaptic activity), to induce the same type of changes in synaptic strength as would be predicted by these theories. The important question which remains is: does this machinery fulfil the same role in a natural setting, under conditions in which the activity to be processed carries real information about the outside world?

1.4 The hippocampus and spatial learning

Discussion of the possible functional role of an LTP-like process in the hippocampus is hampered by a lack of knowledge of the precise function of this structure. The role of the hippocampus in learning and memory is the subject of intensive research and a great deal of debate, much of it heated. One problem is that it may perform different functions in animals and humans, or at least possibly a more complex function in the latter (O'Keefe and Nadel, 1978). Experimental results have therefore been somewhat contradictory and a consensus is proving hard to find: however, in the rat there is some general agreement emerging that part of what the hippocampus does forms at least part of what is necessary for an organism to be able to learn to navigate its way around a new environment.

The physiological investigations which have been conducted to date on the role of hippocampal synaptic plasticity in learning are interwoven with behavioural studies of the learning function of the hippocampus. Any experiment designed to investigate the functional aspects of synaptic plasticity will partly derive its conclusions from the results of the behavioural studies, as well as contributing to those conclusions. For this reason both classes of investigation are described below. The present discussion will focus first on current evidence, including anatomical, psychological and electrophysiological, that the hippocampus has a role in spatial learning. Second, the literature concerning more specific experiments designed to determine whether synaptic plasticity is playing a part in these learning functions will be reviewed in order to set the scene for presentation of the experiments to be described in this thesis.

1.4.1 Two theories of hippocampal function

Many theories of hippocampal function share in common the underlying idea that the hippocampus plays a role in the formation of representations which require the integration of multiple cues across space or time. In the late 1970s two prominent and competing theories of hippocampal function were advanced by Olton and colleagues (Olton *et al.,* 1979) and O'Keefe and Nadel (1978). These were the working memory theory and the cognitive map theory, respectively. Most other hippocampal theories embrace a more general type of learning of which spatial learning is claimed only as a subset (*e.g.* Hirsh, 1974, Rawlins, 1985, Schmajuk and Moore, 1988, Sutherland and Rudy, 1989). Discussion will be restricted to these two models because they have generated a series of spatial experiments the results of which are relevant to those to be presented here.

Working memory theory postulates that the function of the hippocampus is to act as a temporary buffer for storage of information which is only transiently useful to the animal (Olton and Samuelson, 1976, Olton *et al.,* 1979). "Working memory" includes memory for variable environmental features such as the location of food, but excludes invariants such as the general topography of the environment or the fact that food may sometimes be found there, a class of information which the researchers termed "reference memory". Cognitive map theory postulates that the hippocampus has an exclusively spatial function, which is to form and store a metric representation of the environment which can be used to navigate and retrieve information about desirable locations (*e.g.* food sources) within it.

In investigating whether working memory or cognitive map theory more successfully predicts the deficits shown by animals with lesions to part or all of the hippocampus, three types of behavioural task have primarily been employed: the radial maze, the circular platform and the watermaze. Because of their importance these three tasks will be described in some detail, particularly the watermaze, which is the task used in the experiments in the current study.

The radial maze task

The radial maze task (Fig. 1.6A) was designed by Olton and colleagues to test their working memory hypothesis. Working and reference memory are investigated in the radial arm maze as follows. A rat is placed onto the centre of a wheel-shaped maze from which radiate several (usually 8 or 12) spokes. At the end of each spoke, hidden from view, is a piece of food which the animal must learn to retrieve in the minimum possible time. Because in the standard version of the task the food is not replaced after the animal has found and eaten it the optimal strategy is to visit every arm once and no arm more

than once. This type of strategy is known as a "win-shift" rule because the animal must learn to make a different response following every reward. Olton and colleagues found that normal rats solve this task not by systematically moving from one arm to the next, as a human might do, but by choosing the order of arms apparently at random. In order to do this without redundancy the animal must keep a count of which arms it has visited so as not to return to them. This keeping count is the function of working memory. Variants of the task have been devised so as to test other memory functions such as reference memory or non-spatial memory. For example, if only four of the eight arms are ever baited then during a trial a rat has to remember two types of information: which arms usually contain food (reference memory) and which of these it has visited during a given trial (working memory).

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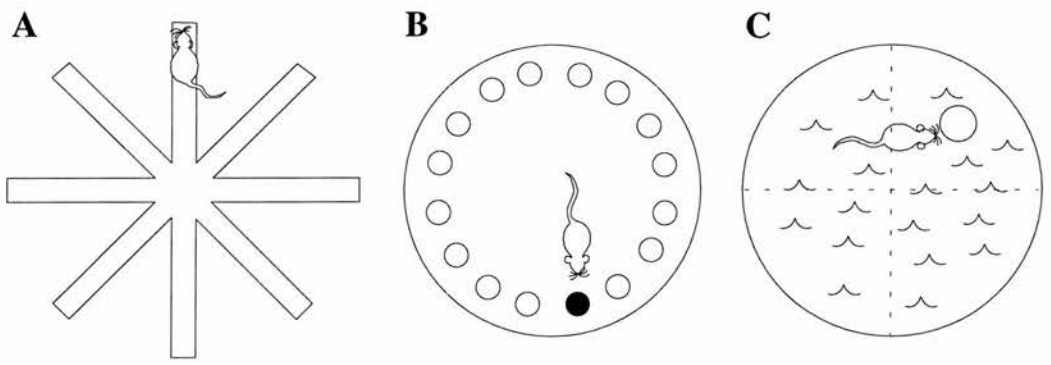


Figure 1.6 (A) Olton's radial arm maze. (B) The circular platform. (C) The Morris watermaze

Several studies have shown that lesions to the hippocampus or some of its subfields selectively impair components of the radial maze task, although there is some disagreement as to the precise nature of the impairment. Olton and colleagues found that animals in which the hippocampus had been de-afferented or de-efferented failed to learn the standard 8-arm task but resorted to visiting the same small subset of arms repeatedly (Olton *et al.,* 1978). Jarrard (1978) found that aspiration lesions of CA1 produced an anterograde but not a retrograde impairment of learning on the task, whereas lesions to the whole hippocampus appeared to affect retrieval of preoperatively learned information as well as disrupting new learning. Nadel and MacDonald (1980) found that hippocampal lesions appeared selectively to affect the spatial component of this task, because when the correct arms were signalled by cues rather than spatial location within the room the lesioned animals performed as well as controls: in other words, non-spatial working memory appeared intact. Rasmussen *et al.* (1989) tested both spatial and non-spatial reference and working memory in rats in which the hippocampus had been deafferented by electrolytic lesions to the entorhinal cortex. In the non-spatial version of the task, the

rewarded arms were signalled by visual and tactile cues and rearranged between trials where necessary, so that rats could not use a spatial strategy to solve any of the tasks. They found that lesioned rats performed as well as controls on both the reference and working memory versions of the non-spatial task but were severely impaired on both types of spatial task. The finding common to all these studies appears to be that lesions to parts of the hippocampal formation produce an impairment of new spatial learning. It remains to be established whether other types of learning may be involved as well.

Another class of studies has investigated the effects of disruption of hippocampal function without making permanent macroscopic lesions. These experiments involve the use of electrical stimulation of hippocampal circuitry and are such are empirically related to the synaptic plasticity experiments to be discussed in the next section. Conceptually, however, they are more closely allied to the lesion studies and may be thought of as producing short-lived functional lesions. Collier *et al.* pre-trained rats on an eight-arm radial maze task using the standard win-shift rule and then investigated the effects of mild electrical stimulation of the hippocampus on the learning of a subsequent "win-stay" rule on the same apparatus (Collier *et al.*, 1982, 1987). In a win-stay paradigm the animal has to learn to make the same response as the one which previously elicited a reward: in this case, to return to an arm which had previously contained food. The authors found that low-intensity electrical stimulation of the dentate gyrus or CA3 and CA1 pyramidal cells at 60 Hz, which was below the frequency needed to induce LTP, produced an anterograde impairment of performance on the task lasting for approximately 1 min (but less than 5 min). Dentate stimulation also produced a retrograde impairment lasting up to 20 min. Knowlton *et al.* (1989) found that seizure-inducing hippocampal stimulation affected performance on a working memory radial arm maze task but not on a reference memory two-arm (T) maze. In the sample phase of the working memory task rats collected food from six arms, before being removed for the application of stimulation. In the choice phase they then had to retrieve the remaining food from non-visited arms. After stimulation the rats made retroactive errors (visiting arms they had already visited during the sample phase) but very few proactive errors (re-visiting arms during the choice phase), suggesting that the stimulation was not producing a confusional state or affecting motivation. The same rats were able to learn which of the two arms in the T-maze always contained food, even when stimulated only 1 min after each trial. Thus the working memory impairment is unlikely to be due to a generalised retrograde amnesia unless the absence of impairment in the T-maze task is simply due to a floor effect (*i.e.* the task is too easy to reveal an impairment).

The circular platform task

The circular platform maze devised by Barnes (1979: Fig. 1.6B) is a large brightly lit disk with several holes around the perimeter, one of which leads to a safe dark haven. A rat placed on the surface near the centre must learn the spatial location of the escape hole. The task resembles the radial arm maze in that the animals must learn to use distal room cues to guide their navigation. It differs from it in that it is aversive (rats dislike bright light and seek darkness wherever possible) and the path of the rat over the surface of the disk is unconstrained. Barnes found that as learning progresses the search pattern of the rat changes from searching randomly to searching hole-by-hole to heading directly towards the goal. Performance is scored by two measures: time taken to find the target escape hole and number of errors made (wrong holes investigated). The effect of lesions of the hippocampal formation on performance of this task has not been investigated: however, it would be predicted that these animals would show a deficit on this task. The effects of disruption of LTP induction have been tested and will be discussed in detail in the next section.

The watermaze task

One of the principal claims of cognitive mapping theory is that not only are salient features of an environment (landmarks) learned and stored but also the spatial relationships between them, so that an animal can use the information to compute trajectories through parts of the environment it has never previously visited. According to this scheme, spatial learning occurs independently of reward (unlike other types of learning such as simple conditioning) and is very rapid. On the basis of single unit recording studies demonstrating the existence of pyramidal cells in CA1 and CA3 whose receptive fields appear to be governed by the spatial location of the rat ("place cells"), O'Keefe and colleagues have proposed that the hippocampus is the brain structure which mediates this type of learning (O'Keefe and Dostrovsky, 1971, O'Keefe and Nadel, 1978). Cognitive map theory predicts that animals with lesions of the hippocampus will be able to solve a task requiring the use of cues only if the cues do not possess a solely spatial relationship to the reward.

The watermaze task (Fig. 1.6C) was developed in order to test some of the predictions of cognitive map theory (Morris, 1981, 1984). The apparatus consists of a circular tank of slightly warmed water made opaque with the addition of a clouding agent such as milk powder or paint. The only means of escape from the water is a platform whose top lies just beneath the water surface and which is invisible unless seen directly from above. The pool itself is made featureless as possible, and the only obvious landmarks are those outside the watermaze in the surrounding room. A rat placed in the water learns, within

only a few trials, to swim to the hidden platform and climb onto it. It can be shown by shifting the platform that the animal is not able to locate it on the basis of local cues such as smell or vision, and because the rat is placed in the pool from random starting positions it is not able simply to remember a sequence of body movements (but see Sutherland *et al.*, 1987). Therefore, its only cues must be the distant cues in the room. Because care is taken to ensure that none of these directly signpost the platform it is likely that the easiest means of solving the task is to form some representation of where the platform is in spatial relation to the cues, which is the function that cognitive mapping theory assigns to the hippocampus. In support of the theory, lesions to the hippocampus profoundly impair watermaze performance (Morris *et al.*, 1982, Schenk and Morris, 1985, Sutherland and Rudy, 1988, Morris *et al.*, 1990). This can be demonstrated using several measures of spatial localisation, of which two are commonly employed. First, when the platform is present the time taken for normal rats to locate it and climb onto it falls rapidly across trials until the rats are reliably swimming straight to the platform location from any starting position. Rats with hippocampal lesions also show a significant improvement in time taken to find the platform but observation of their swimming path shows that at no time do they swim straight towards it. Rather, they swim in circles at an appropriate distance from the wall until they run into it "by accident" (Morris *et al.*, 1990). This apparent lack of specificity of search can be quantified using the second performance measure. If the platform is removed from the pool after a normal rat has learned the task and the rat is then allowed to search freely, it swims directly to the platform's previous location and then concentrates on searching that quadrant of the pool. A high percentage of the total time the rat spends in the pool during that trial will be spent in the training quadrant. A rat with a hippocampal lesion will use its previous strategy of swimming in circles rather than heading towards a specific region of the pool and will therefore distribute its search equally among all 4 quadrants of the pool.

The striking deficit of watermaze performance seen in rats with hippocampal lesions has been one of the strongest influences behind the now widespread acceptance of the importance of an intact hippocampus for spatial learning. Although a full description of the nature of the deficit is still lacking, the watermaze task provides a convenient means of assessing hippocampal function.

1.4.2 Synaptic plasticity and spatial learning

The spatial learning function of the hippocampus has provided a useful backdrop against which to set studies of the contribution (if any) of synaptic plasticity to learning. This is largely because synaptic plasticity is so prominent and easy to study in the hippocampus, a fact which more than compensates for the difficulties to date in completely determining what the hippocampus does. In addition, as studies of synaptic plasticity become more

advanced they are in turn *contributing* to the formation of theories of hippocampal function. A bootstrapping approach like this is somewhat arduous but provides a wealth of data which may also prove to be applicable to other brain structures. The remainder of the introduction will be devoted to reviewing the progress which has been made in elucidating a role for hippocampal synaptic plasticity, and to outlining the rationale for the experiments to be presented in the remaining chapters.

Testing the synaptic plasticity/learning hypothesis

The formulation of a hypothesis involves the creation of a postulated network of causes and effects which can then be tested. If the causal network is correct then successful predictions can be made about the effects of one of its elements on other, connected elements. If the predictions fail then the hypothesis must be modified or discarded.

In the synaptic plasticity/learning hypothesis the causal chain is thought to be as follows:

learning experience→synaptic strength changes (natural LTP)→learning & memory

In LTP induction the causal chain looks like this:

tetanisation→synaptic strength changes (LTP)→increased evoked field potential

A possible scheme of how the synaptic plasticity/learning relationship interacts with LTP is shown in the diagram below (Fig. 1.7). The experimenter has information available about the elements enclosed in boxes. The links represented by solid lines represent what is known with a reasonable degree of confidence: the dotted lines represent the hypothesis under consideration, that synaptic plasticity governs naturally occurring synaptic strength changes which mediate (or rather which *comprise*) learning and memory.

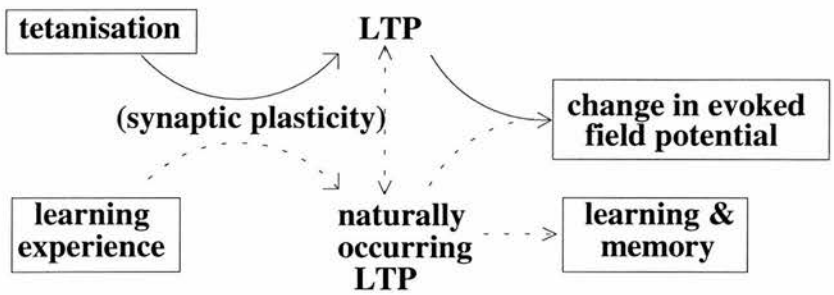


Figure 1.7 Scheme of postulated relationship between LTP and learning. The boxes enclose factors accessible (by observation or manipulation) to the experimenter. In a behaving animal the remainder are inferred or studied using *in vitro* techniques after learning is completed. The dotted line connecting LTP and naturally occurring LTP represents the assumption that both types of synaptic strength change take place on a common set of synapses.

If the proposed scheme is correct, then correlations should be observed between factors which are causally connected in the chain. Certain possible correlations are obvious and

undisputed: for example, that between a learning experience and the formation of a memory, or between tetanisation and the increase in field potential size. The correlations of interest involve those between links in the upper and lower causal chains: for example, between learning and change in evoked potential size, or tetanisation and memory formation.

Other targets for experimental investigation are the common links between the learning chain and the LTP chain, which are (1) synaptic plasticity and (2) the assumption that both LTP and naturally occurring LTP take place on the same set of synapses. With reference to the above diagram, the various types of experiment can be classified as follows (see also Morris and Baker, 1984):

(1) Effect of learning experience on the evoked field potential,

(2) Effect of learning experience on LTP,

(3) Effect of tetanisation on learning and memory,

(4) Effect of manipulating synaptic plasticity on learning and memory,

(5) Correlation between evoked potential change and learning and memory, and

(6) Collapse of learning experience and tetanisation to a single event.

Effect of learning experience on the evoked potential

The synaptic plasticity/learning hypothesis postulates that synaptic strength changes occur as a result of a learning experience, these changes constituting memory formation. If this is so then enhancement of the evoked field response might be expected, since synaptic efficacy is known to be one of factors contributing to the size of the response. This prediction has been tested several times. Sharp *et al.* (1985) kept five rats in a restricted environment for several weeks, to allow synaptic strengths to "decay" to their lowest possible levels. They then exposed three of the rats to a rich and spatially complex environment and observed an increase in the size of the population spike with a smaller increase in two animals in the size of the EPSP (the EPSP in the third animal declined). A subsequent study showed that this enhancement of the population spike decayed more quickly in old animals (Sharp *et al.*, 1987), with time constants for young and old animals similar to those which have been observed following electrically induced LTP (Barnes, 1979; see below). From these observations the authors suggested that the population spike increase might reflect the naturally-occurring increase in synaptic efficacy hypothetically needed to store the newly acquired spatial information. However the phenomenon differed from electrically induced LTP in that there was little (first experiment) or no (second experiment) change in the size of the EPSP.

The picture became more complicated with further studies investigating the effects of exploration on the evoked response. It had previously been noticed that over the 5 min

after being replaced in a recording chamber (which could be considered a novel environment) the size of the EPSP increased slowly (Barnes, 1979). This phenomenon was examined in more detail by Sharp *et al.* (1989) who transferred rats between several different environments while recording the evoked responses. Their findings were that while brief handling in the home cage produced very small increases in the EPSP, removal from the home cage and placement in a recording chamber produced substantial EPSP increases (around 30%) developing over 15 min and lasting up to an hour after replacement in the home cage. The population spike showed a corresponding decrease. The amounts of EPSP increase and spike decrease correlated highly with a quantitative measure of prior exploration. Green *et al.* (1990) showed that treadmill running of the rats was associated not with a rise but rather with an abrupt fall in the size of the EPSP, a result which appeared to indicate that motor activity alone could not explain the exploration-associated changes. Finally, Croll *et al.* (1992) showed that like LTP, exploration-evoked changes were abolished with the NMDA receptor antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine maleate (MK-801).

Again, these findings differed from electrically-induced synaptic efficacy changes in several puzzling ways. First, the EPSP and population spike appeared to vary inversely. Second, abrupt decreases of the EPSP were provoked by certain manipulations such as the treadmill-running condition. Third, although exploration-associated changes were evoked when an animal was placed in a new environment, subsequently placing it in a second new environment failed to produce any further changes, even though the synaptic plasticity model would have predicted additional storage of new information and hence a further increase in EPSP slope.

A possible underlying reason for these contradictory findings was recently advanced by Moser *et al.* (1993). Puzzled by observations that learning of a spatial task in the watermaze was accompanied by substantial *decreases* in EPSP size, these authors conducted a careful study of brain temperature during different behavioural activities and found unexpected and large changes in temperature following apparently trivial physical exercise. An increase in hippocampal temperature was very highly correlated with an increase in EPSP slope and a decrease in population spike height – exactly the changes associated in the above studies with exploration. Treadmill running in rats acclimatised to the new environment produced large increases in both brain temperature and EPSP size. However, when non-habituated rats were used the EPSP showed an abrupt fall, as in the Green *et al.* (1990) study. It thus appears that many previously reported learning-associated changes in evoked field potentials may be seriously confounded by behavioural effects. Dissociating the possibly subtle changes in plasticity-related evoked field potential size from the large non-specific changes produced by associated

behaviours and affective states presents a formidable task, at least in the domain of field potential recording.

Similar retrospective difficulties of interpretation plague other behaviour/evoked-potential studies. For example, in an experiment by Rüthrich *et al.* (1982) rats were trained to learn to escape a footshock by running into the brightly lit arm of a Y-maze, and the dentate population spike was measured immediately before and then 1 min, 4 h and 24 h after training. Rats which were pseudoconditioned showed an initial non-significant decrease of the population spike which by 24 h had turned into a slight, also non-significant increase. Conditioned rats showed no change initially but both the 4 h and the 24 h measures were up to 40-50% larger. When the conditioned rats were divided into good and poor learners the poor learners showed less change than the good learners. Studies such as these suggest the interesting possibility that the increase in the population spike is the result of increases in synaptic efficacy mediating the learning of the task: however, they must now be re-examined in the light of the temperature findings. For example, a possible explanation expressed in terms independent of synaptic plasticity might look like this: with successive recording sessions the conditioned rats became more acclimatised to the recording conditions and the population spike increased with the associated decrease in exploratory activity, as in the exploratory modulation studies discussed above. However, rats in the pseudoconditioned group received a different learning experience because they were subjected to series of unpleasant footshocks over which they had no control. The relevance of uncontrollable reinforcement to studies of evoked potentials and LTP will be discussed later, in the context of stress effects on LTP induction. In addition, the poor learning rats in the conditioned group learned only partial control over the shocks and hence also presumably received more of them than the good learning rats. If the effects of shock-associated stress are to reduce the rate at which the animals acclimatise to the recording conditions the result might be a failure in the unconditioned or poorly conditioned animals of the population spike to return to its normal (habituated) state, rather than an information-storage-associated increase in the conditioned animals as the authors suggested. A similar explanation could account for the findings of Reymann and Ott (1983) that simple unconditional footshock administration results in an increase in the size of the EPSP.

Skelton *et al.* (1987) conducted a related type of study using an appetitive rather than aversive task. This experiment produced an interesting finding which is less easy to explain in terms of behaviour-associated temperature changes. A group of five rats was trained on an operant conditioning task (food delivery, in response to a tone, which was expedited if the rat touched the food hopper during the tone) for 8 days followed by free feeding in a different apparatus for a further 8 days. A second group of five rats was given the same treatment but with the conditions reversed. Evoked dentate population spikes

were measured with daily IO curves conducted 2 h *before* behavioural training on each day, to eliminate confounding behavioural variables introduced by performance of the task. For all rats the conditioning phase alone was accompanied by an increase in the size of the population spike of 20-25%. Ten days after the last behavioural testing day (in other words, 18 days after the last conditioning session for the group trained first and 10 days later for the group trained second) the population spike was still enhanced. The authors interpreted these findings as suggesting that conditioning induced a change in synaptic strength (possibly reflecting information storage of the task) which remained stable unless a new behavioural condition (such as free feeding) was introduced, at which point the synapses returned to their baseline. However, examination of their data in light of recent heightened concerns about behavioural confounding suggests that the explanation may be somewhat more complex.

It was reported that while conditioning was associated with an increase in the population spike, the free feeding condition produced a different effect depending on whether or not a training-induced spike potentiation was present. In the group which had already received conditioning the population spike returned over the first three free-feeding days to its baseline size. In the group for which free feeding was the first behavioural condition the population spike fell to considerably below baseline (*e.g.* see their Fig. 5E). While the first finding was suggested by the authors as representing an active erasure of the synaptic potentiation gained during training, the second was not discussed at all, although the fall was of the order of 10%, a not insubstantial decline given that potentiation was only around 20% (although it was not statistically significant). An explanation of their results must therefore address two points: (1) why did the population spike increase during the conditioning phase, and (2) why did it decrease (in *both* groups) during the free feeding phase? A simple explanation in terms of synaptic efficacy changes in response to information storage does not suffice to explain both unless complicated arguments concerning previous enhancement are invoked.

One possibility is that IO curve measurement over several days produces a gradual decline in evoked responses, irrespective of whether they were potentiated. Another is that the fall of spike size seen in rats in the free feeding condition represents an exploration-associated change. It is somewhat difficult to imagine why 2 h *before* training, rats due to receive free feeding would be more exploratory than rats due to receive conditioning. However, recent lessons are that failure of imagination is not a determinant of the truth or falsity of hypotheses. One intriguing possibility is that the animals were generalising from the apparatus in which they were to receive free food, a Plexiglas chamber, to the chamber in which the recordings were made 2 h beforehand – also a Plexiglas chamber, although somewhat larger with a floor made of wire mesh instead of brass rods. In the conditioning phase food was only signalled by the tone. In the

free feeding phase, by contrast, food was available unconditionally. It could be that during the free feeding phase hungry rats placed into a Plexiglas chamber with no tone present expected to find food and hence began searching for it, prompting an exploration-associated fall in population spike height.

Bergis *et al.* (1990) measured dentate evoked potentials 2 days after aversive training (a tone-footshock association). A very similar pattern of population spike changes was seen, with increases of around 20% in conditioned animals and decreases of around 10% in pseudoconditioned animals. These effects were not statistically significant and attributed to chance by the authors. Nevertheless, there appears to be a certain consistency developing in the literature with regard to post-training evoked potential changes. What they actually mean is another matter. It is clear that these complicated issues will not be easy to resolve using only field potential recording, which is susceptible to many kinds of modulating influence unrelated to information storage *per se* (although possibly contributing to it).

One alternative approach is to use *in vitro* techniques in which the momentary behavioural state of the animal becomes irrelevant (although not necessarily that immediately preceding sacrifice). Green and Greenough (1986) housed rats in a complex environment and subsequently investigated evoked responses in the perforant path/dentate gyrus synapse in hippocampal slices taken from these animals. When the slices were investigated immediately after complex housing an increase was found in both the EPSP and population spike, although unlike LTP there was no change in the relationship between them (*i.e.* no E-S left shift; see Chapter 2). This increase appeared to be independent of changes in general cell or afferent fibre excitability. When the rats were rehoused in impoverished conditions (standard laboratory cages) for the 3 weeks prior to sacrifice the responses did not differ from those of rats which had always been housed in this manner. The use of this type of *in vitro* technology circumvents many of the problems associated with the study of intact animals, including behaviour-associated temperature effects and arousal and stress responses. Because there is no ascending modulatory circuitry remaining it can be stated with a much greater degree of confidence that the change underlying the evoked potential increase is localised to the hippocampus. Whether or not the change is related to synaptic plasticity remains to be determined. It may be that the different experience of the two groups of animals resulted in long-term endocrinological changes such as receptor up- or down-regulation.

Effect of learning experience on LTP

If the synaptic plasticity/learning/LTP relationship depicted in Fig. 1.7 is correct then an association might be predicted between a prior learning experience and the subsequent induction of LTP, even in the absence of an effect on the baseline evoked potentials

themselves. The mediating link in this case is postulated to be synaptic plasticity. In other words, if a learning experience were to modulate such plasticity, for example by altering NMDA receptor numbers or increasing or decreasing the second messenger systems involved in LTP expression, then this might not show up as a change in hippocampal physiology until tetanic stimulation is applied.

It was mentioned in the preceding subsection that Bergis *et al.* (1990) measured the effects of aversive conditioning on the dentate evoked response. These authors then went on to induce LTP in the same animals and found a considerable enhancement of LTP magnitude in the conditioned animals as compared with the pseudoconditioned group. One possible explanation is that the effect results from the small population spike increase seen in the conditioned animals. A second possibility, favoured by the authors, is that synaptic plasticity is increased by training (possibly by effects on the NMDA receptor) resulting in enhanced LTP.

A third possibility has recently been suggested by the findings of several experiments in which animals are subjected to uncontrollable stress prior to LTP measurement. Foy *et al.* (1987) found that if rats were kept in restraining tubes for the 30 min prior to sacrifice, their hippocampal slices subsequently showed considerable less LTP than those from unrestrained controls, suggesting that stress may affect synaptic plasticity. Shors *et al.* (1989) have found that application of electrical footshock in a shuttlebox does not impair LTP if animals are able to escape the shock at will, but profoundly impairs it in yoked controls which are subjected to the same shocks over which they have no control. This effect appears to be mediated by adrenal medullary hormones, possibly opioids (Shors *et al.,* 1990). The similarity between the above Bergis *et al.* study and the latter is obvious: pseudoconditioned animals in that experiment were being subjected to an apparently random series of footshocks whereas the conditioned animals, although being unable to control the shocks, were at least being given warning prior to their administration. Thus, difference in LTP levels between pseudoconditioned and conditioned animals may in fact be explained by a stress-related LTP impairment in the former, resulting from the random and capricious nature of the electric shocks. It appears therefore that investigation of the effects of prior learning on LTP induction is subject to the same pitfalls as the field potential experiments in the preceding section: that is, it is almost impossible to dissociate the effects on LTP of the laying down of the memory trace, wherever that may occur, from the concomitant changes in the behavioural and affective state of the animals.

Effect of tetanisation on learning and memory

The symmetry of the synaptic plasticity/learning/LTP scheme suggests the reverse type of experiment to those described above: namely, investigation of the effects of tetanisation on learning and memory. There are two connecting links between the LTP causal chain

and the learning and memory causal chain: first, both depend on the underlying plasticity of synapses, and second, both are assumed to take place on the same set of synapses. Bearing in mind that synaptic plasticity can be dissociated from the actual synaptic changes themselves, tetanisation might therefore interact with memory formation in two corresponding ways: it might affect plasticity, for example by inducing changes in NMDA receptor function such as those postulated above to mediate learning-induced LTP changes, or it might affect the subsequent occurrence of naturally occurring LTP. To date, no investigations have explicitly addressed the question of whether post-tetanisation plasticity changes might affect learning, so discussion will be restricted to the second interaction.

If tetanisation affects the occurrence of naturally occurring LTP it could be either by facilitating or occluding it. Facilitation might come about if the important factor was total synaptic strength but not the details of its distribution across the synaptic population. For example, if a pathway was simply relaying information from one brain structure to the next without processing it (or, at least, without processing it *synaptically*) then a generalised increase in the strength of its synapses might improve the performance of functions which normally use that pathway. A tetanisation-induced improvement in performance was found by Berger (1984) in a non-spatial task, the conditioned eyeblink response, in rabbits. In this experiment unilateral perforant path tetanisation was found to accelerate acquisition of the eyeblink response to a tone signalling an airpuff. This finding is somewhat puzzling because lesions to the hippocampus do not appear to affect performance on this task (Solomon and Moore, 1975). It could be the case, as Berger suggested, that the role of the hippocampus in this task is to act as a general modulatory agent and that the effect of potentiating the synapses was simply to turn up the gain of the signal. According to this scheme, the hippocampus is not essential to performance of the task but is normally involved, and whereas its destruction does not affect the task (perhaps the signal is then routed elsewhere), its facilitation may improve performance.

Most computational models, by contrast, would predict occlusion of learning after tetanisation of a pathway upon which that type of learning was dependent. Occlusion occurs when two phenomena share a common mechanism so that the occurrence of one interferes with the simultaneous or subsequent occurrence of the second. Occlusion studies are often used in physiology to establish whether such common mechanisms are involved. For example, if stimuli are applied to two afferent neural pathways simultaneously, the finding that the evoked response is smaller than the sum of the separate responses evoked by stimulation of each pathway separately strongly suggests that the two pathways terminate on an overlapping set of target cells. Occlusion of learning and memory formation by LTP induction would constitute very strong evidence that both phenomena involved the same set of synapses. Since it is known that LTP

comprises a change in synaptic strength, the finding that it occluded memory formation would extend the interpretation to suggest that learning itself is also based on a change in synaptic strength.

Two prominent studies have suggested that occlusion of spatial learning by LTP induction in the dentate gyrus is indeed readily elicited (McNaughton *et al.*, 1986, Castro *et al.,* 1989). Since then a number of studies have called this finding into question, including two experiments in the present thesis. Because most of these latter studies were published together during the course of conduction of the experiments to be presented here (see *Hippocampus,* **3(2)**), they will be discussed in detail in later chapters. For the time being, only the original two experiments will be presented.

The first study, conducted by McNaughton *et al.* (1986), tested spatial learning of rats in two tasks: the circular platform task and an eight-arm radial maze. First, rats were trained to learn the location of an escape hole on the circular maze. In 12 animals LTP was then induced bilaterally in the perforant path/dentate gyrus synapses while in the remaining 12 only low frequency pulses were applied. The following day the rats were tested on a reversal of the task, where the goal was shifted almost (though not quite) to the far side of the maze. The first trial of this reversal phase thus tested the animals' retention, after LTP induction, of the previous goal location, since the animals had no way of knowing that the goal had shifted. If spatial information was being stored across these synapses it was postulated that LTP induction might erase this information by setting all the synaptic strengths to the same level. However, the rats appeared to have no difficulty in navigating towards the previous goal location, suggesting that their memory and spatial localisation abilities were intact. In subsequent trials the animals were tested on their learning of the new goal location. Animals which had received bilateral perforant path tetanisation made significantly more errors than the low-frequency controls over the remaining several days of training, suggesting a long-lasting disruption of spatial learning following tetanisation.

The experiment was then repeated with 16 new rats but the animals received one trial of the reversed goal location *before* tetanisation, which was commenced 5 min later. The procedure was repeated on the following day and the animals subsequently trained to this new location. The 8 tetanised animals appeared to have learned nothing about the new goal location during the single trial they had received before tetanisation, because they made as many errors on the second reversal trial as they had on the first. Furthermore, in subsequent training trials they showed no improvement in performance whereas the low frequency control animals showed a significant improvement. Thus, LTP induction appeared to have produced an anterograde amnesia lasting several days (at least 5) and a retrograde amnesia lasting at least 5 min. Further analysis suggested that the tetanised animals were persisting in approaching the previous goal location, suggesting as before

that pre-tetanisation memory and spatial localisation ability were unimpaired. In two more experiments it was shown that in addition to reversal learning, new learning was impaired (though not as severely). However, performance on the radial arm maze, a task known to require an intact hippocampus (unlike the circle maze, in which this has yet to be demonstrated) appeared normal.

The second study was carried out in the watermaze and extended these observations (Castro *et al.,* 1989). The methodology of their experiment will be returned to in greater detail in Chapter 3, but a brief description is as follows: 8 rats were given bilateral tetanic perforant path stimulation daily for 14 days in order to drive synaptic strengths to their maximum (although it was not made clear why such "saturation" was thought necessary, since moderate levels of induction appeared to suffice in the circular platform experiment). Half these rats were trained on a watermaze task and their performance compared with that of rats receiving low-frequency pulses only. The tetanised rats showed a profound deficit in their ability to learn the platform position. Two weeks later the remaining tetanised rats were trained on the same task while the originals were trained on a reversal. Both groups performed as well as controls, implying that the deleterious effects of LTP induction had dissipated. The authors suggested that because LTP and learning had returned to normal by 2 weeks post-tetanisation, it was likely that LTP was the cause of the learning impairment. The finding of a spatial learning impairment in rats in which synaptic strength had been saturated provided strong support for the idea that an LTP-like process may mediate learning, and the experiment has been very influential in shaping the LTP/learning hypothesis.

Effect of manipulating synaptic plasticity on learning and memory

Manipulations of synaptic plasticity alter the capacity of synapses to change without necessarily actually effecting any such changes. As such, this type of approach may be distinguished from occlusion experiments like those described above. Synaptic plasticity may be affected indirectly, for example by prior learning experiences such as those discussed earlier, or it may be affected by directly targeting the plasticity machinery itself by means of pharmacological or genetic manipulations which modulate the sequence of events responsible for the induction of plastic changes like LTP. The effect on learning of such manipulations is the source of a great deal of study at present, since the finding of predicted learning changes would support the synaptic plasticity/learning hypothesis, while the absence of such changes in the face of known blockade of plasticity would greatly weaken it.

In order to target synaptic plasticity without affecting normal synaptic function (such as transmission, transmitter re-uptake, postsynaptic membrane potential and so on) it is necessary to isolate those factors that are specific to plasticity and not shared by other

mechanisms. This can never be done with complete confidence, and a possible interpretation of all plasticity-related effects is that some other function was inadvertently affected by a given intervention. However, it may be argued that the greater the number of different approaches undertaken to altering plasticity, the smaller the likelihood that such unwanted side-effects are confounding the results, because it becomes probabilistically less likely that the same mechanisms were repeatedly affected by different manipulations, except for the one which was deliberately targeted. For this reason nearly every link in the chain between pre/postsynaptic conjunctive activity and LTP expression has been investigated to assess its effects on learning. These are the NMDA receptor, second messenger systems and RNA and protein synthesis.

Because the NMDA receptor is critically involved in LTP induction and does not appear to play a part in normal synaptic transmission (Coan and Collingridge, 1985) it is an ideal candidate for a plasticity blocker with which to test the plasticity/learning hypothesis. Only studies investigating spatial learning will be considered here. The first and most prominent of these were conducted by Morris and colleagues in the watermaze (Morris *et al.,* 1986, 1989, Morris, 1989, Davis *et al.,* 1992) using rats in which the NMDA receptor blocker amino-5-phosphonopentanoate (AP5) was infused into either the cerebral ventricles or the hippocampus itself. In various experiments it has been shown that rats receiving NMDA receptor antagonists under a variety of protocols could not learn either the task itself or a reversal, showing longer latencies to find the platform during training and little specificity of search on the absent-platform test. Studies using the competitive NMDA antagonist MK-801 have found a similar effect on watermaze performance in rats (Robinson *et al.,* 1989) and gerbils (Mondadori *et al.,* 1989). Using this drug, Shapiro and Caramanos (1990) have also found a deficit on reference but not working memory on the radial arm maze 4/8 task. An interpretation of these experiments is therefore that NMDA receptors are being used in spatial learning (possibly formation of a spatial map).

These types of study are not without problems, as Keith and Rudy (1990) have (somewhat pointedly) noted. Although NMDA receptors are found most abundantly in the hippocampus, they are also found elsewhere in the brain (Monaghan and Cotman, 1985) and injection of the drugs into the peritoneum or infusion into the cerebral ventricles would allow them to exert their effects on other brain structures. Thus, it cannot be claimed on the basis of these results that synaptic plasticity in the hippocampus is necessarily responsible for spatial learning, as distinct from plasticity elsewhere. Furthermore, NMDA receptors are involved in other synaptic functions than just plasticity. Such functions within the hippocampus itself include modulation of complex spiking of pyramidal cells (Peet *et al.,* 1987, Abraham and Kairiss, 1988) and theta rhythm (Leung and Desborough, 1988). Outside the hippocampus the NMDA receptor appears to play an important role in many processes: for example, modulation of sensory

input (Salt, 1986), anxiolysis (Clineschmidt *et al.,* 1982), neural development (Kleinschmidt *et al.,* 1987) and many others (for review, see Daw *et al.,* 1993). Impairment of performance on a behavioural task following administration of an NMDA receptor antagonist may therefore have many causes. Several attempts have been made to address these difficulties: for example, infusion of antagonist directly into the hippocampus (Morris *et al.,* 1989) or administration of control non-spatial tasks to check that the deficit is specific to spatial learning and not part of some more general disturbance of function (Morris *et al.,* 1986, Morris, 1989). Although there is no doubt that animals are severely impaired on spatial learning tasks, the question of whether the impairment is specifically related to the effects of the drug on synaptic strength changes is still unresolved.

A new approach to modulating synaptic plasticity which aims to circle round some of the side-effect difficulties is the development of transgenic animals whose synapses are congenitally unable to support LTP, while being otherwise apparently normal. The advantage of using a transgenic animal is that complicated and messy administration protocols do not need to be devised, since no drug is being used. For example, it has recently been shown that mice unable to express a subunit of calcium-calmodulin-dependent protein kinase II (CaMK II), also known as "CaM kinase mutants", are impaired on a watermaze task (Silva *et al.,* 1992a). Slices made from their hippocampi also do not express LTP (or at least do so only erratically, Silva *et al.,* 1992b). This enzyme is a critical link in the postsynaptic second messenger chain culminating in LTP expression, suggesting yet again a link between synaptic plasticity and learning. Mutant mice unable to express one of the genes (*fyn*) coding for tyrosine kinase show a parallel impairment of both LTP and performance on a watermaze task (Grant *et al.,* 1992).

The technique of studying animals which have been subjected to this type of "molecular surgery" holds considerable promise but the same caveats as above apply: until it can be shown without question that only the hippocampus has been affected and of that, only the plasticity component of its function altered, it can never be claimed for certain that observed deficits in behaviour do not have a cause attributable to some other structure or to some other function. It is doubtful whether such a claim ever could be made with confidence. In transgenic studies such as these an additional problem arises, because animals with altered gene expression since birth may have developed abnormally as well as showing abnormal adult function. This is particularly clear in the *fyn* mutant studies, where the CA3 region of the adult hippocampus was found to possess a highly abnormal architecture, suggesting that even the hippocampal components of the behavioural deficit might nevertheless have a cause unrelated to plasticity.

Correlation between evoked potentials and learning and memory

In the synaptic plasticity/learning scheme, both learning and memory and the change in size of the evoked potential depend on underlying synaptic plasticity and so correlations would therefore be predicted to occur between them. The first such correlation was observed by the authors of the original LTP studies, who noted that the time course of LTP persistence (up to several weeks in the chronically implanted animals) was considerably closer to that required for a memory mechanism than other neurophysiological plastic changes such as post-tetanic potentiation (Bliss and Gardner-Medwin, 1973). An extension of this correlation to variation in learning ability was made by Barnes (1979) in a comparison of LTP and spatial learning in young and old rats. The behavioural task the rats were required to learn was the circular platform task described earlier, after which the rats received several sessions of perforant path tetanisation. The young rats learned the task faster and with fewer mistakes than the old rats. A single subsequent tetanisation session produced the same amount of LTP in both young and old rats but when the tetanisation was repeated daily the old rats showed a slower accumulation, and by the third consecutive tetanisation day had gained considerably less LTP. This appeared to occur because after repeated tetanisation overnight LTP decay became markedly slower in the young rats but remained fast in the old rats. On the basis of these findings Barnes suggested that repetition of the stimulation which evoked plastic changes in the synapses somehow prolonged the time course of those changes, producing an accumulation of EPSP LTP which reflected the amount by which the durability of the plastic changes was increased. The same animals which showed the greatest accumulation of LTP also showed the greatest retention of the spatial task after repeated training trials. She postulated therefore that the durability of hippocampal synaptic plasticity may underlie retention of the spatial task. In a subsequent study, Barnes and McNaughton (1985) further showed that in young and old rats, the ratio of LTP decay between the two groups matched the ratio of memory retention.

Following Barnes, much of the work investigating correlations between LTP and behaviour has continued to focus on comparisons between young and old animals. This is largely because the hippocampus is prominently affected by the aging process, on anatomical, neurochemical and neurophysiological criteria. However, in assessing the contribution of the studies of aging animals to the plasticity/learning hypothesis it is important to bear in mind that age is a highly generalised influence affecting all organ systems and biochemical pathways. Therefore, any comparisons between young and old animals and features of their physiology (such as their LTP parameters) are potentially confounded by the multitude of parallel changes taking place. For example, in addition to possessing less durable LTP, old rats are larger and have poorer eyesight. However there

is no reason to suppose from this "correlation" that LTP has anything to do either with growth or with vision (although it might do). Within-animal correlations are more convincing because it would not necessarily be the case that the same *distribution* of two parameters would hold unless they were somehow functionally related. However even this cannot be asserted with complete confidence: for example, elderly rats in which axonal transport was impaired might show closely matched deficits in both LTP and spatial learning, even if the two were not functionally connected. For this reason the power of age-related correlations to support the plasticity/learning hypothesis comes mainly from their failure to find counter-examples which would disprove the hypothesis under consideration: for example, absent LTP in the presence of perfect spatial learning.

The aging literature is extensive and will not be covered in detail here. However, it is worth mentioning one study in which a strong within-animal correlation was found between the magnitude of LTP induction and spatial learning ability, in young and old rats. Deupree *et al.* (1990) used standard stimulation parameters (50 pulses at 100 Hz) to induce LTP in the hippocampal slices of animals whose spatial learning ability had been determined in a watermaze prior to sacrifice. They failed to find any differences between the magnitude of subsequent potentiation in young and old animals. However, when LTP was induced using weaker stimulation (4 pulses) the old animals showed less potentiation at 1 minute (short-term potentiation, STP). Most importantly from the point of view of the plasticity/learning hypothesis, within-animal correlations of prior spatial performance with both STP and LTP revealed greater enhancement in animals with better spatial learning ability, regardless of age.

Several other shared features have been found between LTP induction and learning. For example, glutamate release from presynaptic terminals in the dentate gyrus is increased after both LTP induction (Bliss *et al.,* 1986) and classical conditioning (Laroche *et al.,* 1987). Both LTP and conditioning are facilitated by post-trial stimulation of the reticular formation (Laroche and Bloch, 1982). Together, correlations such as these suggest that learning and LTP share an underlying mechanism, supporting the hypothesis that synaptic strength changes might also be expected to occur as part of memory formation. However, they do not constitute proof of this hypothesis, because it is always possible that artificially induced synaptic strength changes are an artefact and never occur naturally – or that they occur, but not as part of learning. However, the more it is discovered that LTP and learning ability vary together, and the harder it is to dissociate them, the more support is generated for the hypothesis.

Collapse of learning experience and tetanisation to a single event

Finally, it is worth mentioning some unusual experiments in which the two halves of the synaptic plasticity/LTP/learning scheme have been amalgamated (Fig. 1.8). This is

accomplished by making the tetanisation process itself the conditioned stimulus (CS) in an associative learning paradigm.

For example, Laroche *et al.* (1989) used a perforant path tetanus as the CS to signal footshock in a conditioned suppression task. In this type of task, rats trained to press a lever for food reward learn that a stimulus (in this case the tetanus) signals some type of reinforcement and stop pressing the lever until the event is over. The change in rate of lever pressing is used as an index of how much the rat has learned about what is going to happen next. Laroche *et al.* found that rats in which robust LTP developed learned the task whereas those in which LTP development was blocked, either by subthreshold tetanisation, infusion of an NMDA antagonist or simultaneous tetanisation of convergent inhibitory inputs, failed to do so.

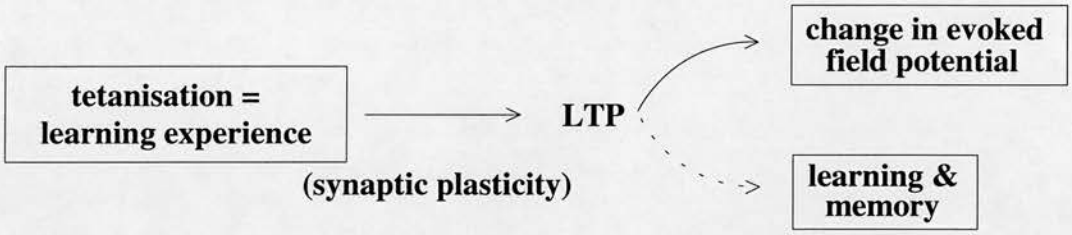


Figure 1.8 The synaptic plasticity/learning scheme when the learning experience and perforant path tetanisation are amalgamated: that is, when tetanisation becomes the CS in a Pavlovian conditioning paradigm.

In a further experiment Doyère *et al.* (1992) showed that retention of the task correlated inversely with the decay rate of LTP, so that rats in which LTP returned rapidly to baseline performed poorly when retested on the task some time later whereas those in which potentiation persisted retained the task well. Thus, manipulation of the first link in the above scheme resulted in a parallel effect on the two end links, suggesting that the scheme may accurately describe some of the processes involved. However, there are some serious criticisms of this type of study which have never been fully addressed by the authors. The most important is that because the CS is producing LTP in the pathway being tested, it is having the effect of strengthening itself. Laroche *et al.* argue that this is entirely reasonable, since that may well be what takes place in any type of learning: that is, one of the input pathways becomes stronger. However, suppose for the sake of argument that in normal learning under natural conditions no changes take place in the perforant path at all – how can these results then be explained? The obvious answer is that rats are learning better about the CS when LTP occurs simply because the CS is getting stronger – it is as if a very faint tone in a tone-footshock task had been made louder for some animals, who naturally would then find it easier to learn about it. Since the result can be explained either with or without invoking the presence of naturally-

occurring perforant path plasticity, no conclusions can be drawn about *normal* learning from the finding of improved learning in animals gaining LTP in this task.

A more convincing case would be made if the CS consisted of low frequency rather than high frequency pulses. The finding of LTP developing when these pulses were paired with a US would be very interesting because LTP would not normally be expected to develop as a result of low frequency pulses alone. Matthies and colleagues used a low frequency of perforant path stimulation (15 Hz) as the CS in a shuttlebox avoidance task on rats, and found an increase in the size of the EPSP in good as compared with poor learners with an associated right-shift of the E-S relationship (Ott *et al.,* 1982, Matthies *et al.*, 1986). The design of this experiment circumvents some of the difficulties discussed above. Unfortunately, it is susceptible to the same type of alternative explanation as in the Y-maze experiment of Rüthrich *et al.* (1992): that is, the poor learners were receiving more shocks and therefore were more stressed than, or behaved differently from the good learners. This type of confounding of behavioural state or change in stimulus salience is extremely difficult to avoid in these types of experiment. A possible approach would be to demonstrate the same result using either an appetitive or an aversive task. The finding of the same result when opposing reinforcers were used would rule out many of the non-specific behavioural and affective confounding factors in these experiments (though not all of them). To date this does not appear to have been attempted.

1.5 Introduction to the experiments

To summarise, then: the hippocampus, a structure which appears to play an important role in the formation of certain types of memory, also readily supports experimentally-induced changes in synaptic efficacy. Since synaptic efficacy changes are fundamental to most computational models of learning, the next task is to discover whether naturally occurring hippocampal synaptic changes, resembling those induced experimentally, normally mediate its learning role. If it should turn out that they are *not* fulfilling this role then either the computational models are wrong in attributing memory formation to synaptic strength changes, or the hippocampus is not participating in memory formation after all, or both. Because either of these possibilities would force a serious re-examination of psychological and computational theories, the question is of considerable importance.

Much experimental effort has been devoted to trying to resolve this issue, and the results to date have been summarised in the preceding sections. Nearly all of the approaches used to date are open to criticisms of various types but so far, the crucial finding which would refute the hypothesis – that is, the finding that animals could show normal learning in the face of complete blockade of synaptic strength changes – is lacking. To the contrary, nearly all the studies have produced evidence which supports the hypothesis. The

experiments to be described in the next five chapters were designed with a view to gathering more evidence regarding the putative role of synaptic plasticity in learning.

The experimental part of this thesis is divided into two sections. Part I is devoted to an attempt to replicate the occlusion studies of McNaughton *et al.* (1986) and Castro *et al.* (1989), where, as discussed above, perforant path tetanisation had been found subsequently to impair spatial learning. The reasons for making such an attempt are discussed in the introduction to these experiments (Chapter 3). The two experiments comprising this section produced two unexpected results, one of which was a failure to replicate the occlusion findings. Possible reasons for this failure are discussed with reference to a number of other contemporaneous studies which also failed to produce an LTP-associated learning impairment.

The second result was that a strong correlation was found between the magnitude of LTP induced in individual animals and their subsequent performance in the watermaze. An exploration of this finding formed the basis of Part II. The possibility was examined that the correlation had arisen because LTP induction had influenced subsequent spatial learning, in the manner of Berger's (1984) finding that LTP induction enhanced conditioning in rabbits. However, it was found that the results of the original two experiments could not be replicated. In a final experiment a possible reason was found for the difficulty in replication which also may explain why other investigators have not previously reported seeing such a correlation in similar experiments: namely, that the correlation reversed sign depending on the strength of the test stimuli used to measure LTP. Using a criterion for LTP measurement that took into account its systematic variation with stimulus intensity, it was found that the correlation between LTP and spatial learning returned, but that it appeared to indicate that poor learning animals were demonstrating *increased* synaptic plasticity. This apparently paradoxical finding is discussed.

Finally, two further aspects of the correlation were considered: first, whether or not it was specific to spatial learning or generalised to other types of learning (in this case, simple conditioning). It was found that although spatial learning ability did not correlate with ability on a conditioning task, nevertheless when LTP induction was compared only for those animals which performed well or poorly at both tasks the correlation increased markedly, suggesting that there may be an interaction between the two types of learning. Second, the possibility was investigated that the correlation could be explained on the basis of the distribution of intra-tetanic NMDA current between animals. Estimation of the contribution of NMDA current to the evoked response during a tetanus was conducted by blocking NMDA receptors and comparing the waveform with that evoked prior to drug injection. There was a positive though non-significant correlation between intra-

tetanus NMDA current magnitude and spatial learning, contrasting with the negative correlation seen with LTP induction. The discussion in Chapter 6 focuses on the implications of the collection of findings as a whole for the synaptic plasticity/learning hypothesis, and suggests a direction for future research.

Chapter 2 – General Methods

Electrophysiological and behavioural methods were similar for all of the experiments to be described in the next three chapters, so for brevity a general description is provided here. Additional details of individual experiments will be introduced in the appropriate sections.

2.1 Subjects and housing

The animals used in this study were male Lister hooded rats (250-400 g), obtained from the department breeding colony. Prior to surgery the rats were housed in groups of 3-5 animals. After surgery both implanted rats and unoperated controls were kept singly except for Experiment 1 where operated rats were fitted with protective caps over the implants and housed in groups of 2-3. Because of indications that the caps might be contributing to electrode shift this practice was discontinued for the remaining experiments.

Rats were maintained on a 12:12 h light:dark cycle with lights on at 0800 h. They had free access to food (Purina rat chow) and water except for Experiment 4, where access to food was restricted for the conditioning phase of the experiment.

All of the experiments were run in replications consisting of between 6 and 14 animals.

2.2 Electrophysiology

2.2.1 Background

The work conducted in this thesis involved the measurement of evoked population responses from dentate granule cells and so before proceeding to a description of the methods used, a review of the measurement technique is presented here.

Measurement of the dentate evoked response

The evoked response following a single electrical stimulus at 300 μA to an electrode situated in the angular bundle of the perforant path is shown in Fig. 2.1. The recording electrode was located in the hilus of the dentate gyrus extracellularly, so the response consists of the summation of the individual responses of all the granule cells which were activated by the stimulated fibres.

Measurement of the waveform consists of calculation of the slope of the rising phase of the EPSP and the height (or rather depth) of the population spike. The first parameter provides an estimate of synaptic efficacy relatively uncontaminated by other influences such as feed-forward inhibition from the basket cells mentioned in Chapter 1 (Lømo, 1971a), although near the visible onset of the population spike it may be partially influenced by the firing of small numbers of granule cells (Andersen *et al.,* 1971a). The slope of the very early part of the field potential is caused by the voltage change resulting from ion channel opening in response to neurotransmitter receptor activation (Abraham and McNaughton, 1984), so a greater synaptic strength will result in greater ion channel opening, faster ion influx and a steeper slope. If inhibitory influences are to be recruited their effect will not be seen until later in the field potential, because of the additional delay introduced by the extra synapse.

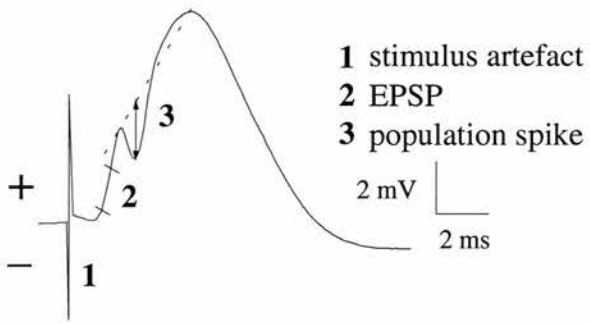


Figure 2.1 Evoked response of a population of granule cells to an electrical stimulus to the perforant path showing the two parameters (EPSP slope and population spike height, 2 and 3 respectively) which are used to describe the size of the waveform. (1) Stimulus artefact produced by the current pulse, followed by a short delay representing axonal and synaptic transmission of the stimulus. (2) Rising phase of the EPSP. The two short lines represent the cursors between which the EPSP slope was calculated by linear regression. (3) Downgoing notch of the population spike. The length of the arrowed line from the tip of the spike to the dotted line drawn between the two local maxima represents the population spike height.

The second parameter, the height of the population spike, provides a measure of how many granule cells are discharging (Andersen *et al.,* 1971a). This is a more complex response because the transduction of depolarisation in the dendrites to axonal action potentials is influenced by factors such as cell excitability (the threshold for action potential firing), inhibition and the time taken for spread of current down the dendrites to the cell soma. If the synapses activated by the stimulus are at varying distances from the soma (as for example in the lateral and medial perforant path terminations discussed in Chapter 1) then their effects will trigger action potentials at slightly different times, resulting in a broader smaller spike. If they are all at the same distance then the action potentials will be more synchronous and the spike will be taller and narrower. For this reason some investigators prefer to use spike area rather than spike height as an estimate of the number of granule cells which fired, although in practice it appears to make little difference (Barnes, 1979). In the present study, spike height is used for ease of

calculation. Data from both EPSP and population spike measurements will be presented throughout.

IO curves

Because of the complexity of the population response evoked by synchronously activating large numbers of perforant path fibres, it is sometimes desirable to record an input-output (IO) curve, which is a series of responses evoked over a range of stimulus intensities (*e.g.* Lømo, 1971b). On the basis of the results to be presented in this thesis it will later be argued that in LTP measurement it is *always* desirable to do so. An example of a typical IO curve (taken from data to be presented in more detail in a later chapter) is shown in Fig. 2.2.

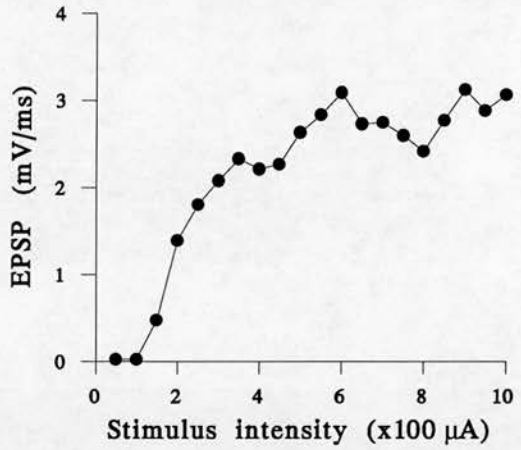


Figure 2.2 A typical example of an input-output curve. The ordinate represents intensity of the current pulses applied to the perforant path in order to evoke single field responses in the dentate gyrus. The abscissa represents the slope of the rising phase of the EPSP.

Relatively little attention has been paid in the literature to the factors responsible for the shape of the IO curve, but some of the influences may be assumed to be as follows. At very low stimulus intensities almost no fibres are activated by the stimulus and consequently very little postsynaptic depolarisation is produced. As the stimulus intensity increases two things happen: first, fibres nearest the stimulating electrode, where the current intensity is greatest, begin to produce axonal action potentials which result in synaptic transmission and postsynaptic depolarisation. Second, the current spreads further outwards from the current source at the tip of the stimulating electrode activating fibres over a wider range. Both of these effects combine to produce increasing postsynaptic depolarisation, resulting in the rising slope of the sigmoid curve seen in Fig. 2.2. The slope of this rise is therefore being governed by several factors. Details of the influences on IO curve shape will be presented in Chapter 5.

Long-term potentiation

The effect of administering a high-frequency train of pulses to the perforant path is shown in Fig. 2.3 A. Before such tetanisation, the response following a single electrical stimulus has the characteristic and reproducible size and shape described above. During a tetanus the evoked response becomes broader and longer-lasting (Fig. 2.3B). After tetanisation the appearance of the waveform is seen to have changed (Fig. 2.3C), the slope of the EPSP becoming steeper and the height of the population spike increasing.

The reason for the broadening of the intra-tetanic waveform is shown in Fig. 2.4. The solid line in the upper part of the figure shows the shape of the waveform normally evoked by a tetanus. The dotted line shows the waveform evoked when most or all of the NMDA receptors have been blocked by *i.p.* administration of the competitive NMDA antagonist 3-[(±)-2-carboxypiperazin-4-yl]-1-propenyl-1-phosphonic acid (CPP-ene). This procedure will be described in greater detail in Chapter 5. The intra-tetanic waveform evoked in the absence of NMDA receptor activity is smaller and briefer, like that evoked during a single pulse. The portion of the normal intra-tetanic waveform which is contributed to by NMDA receptor activity is shown by subtraction in the lower part of the figure. It is the Ca^{2+} component of this potential which triggers the processes required for LTP induction.

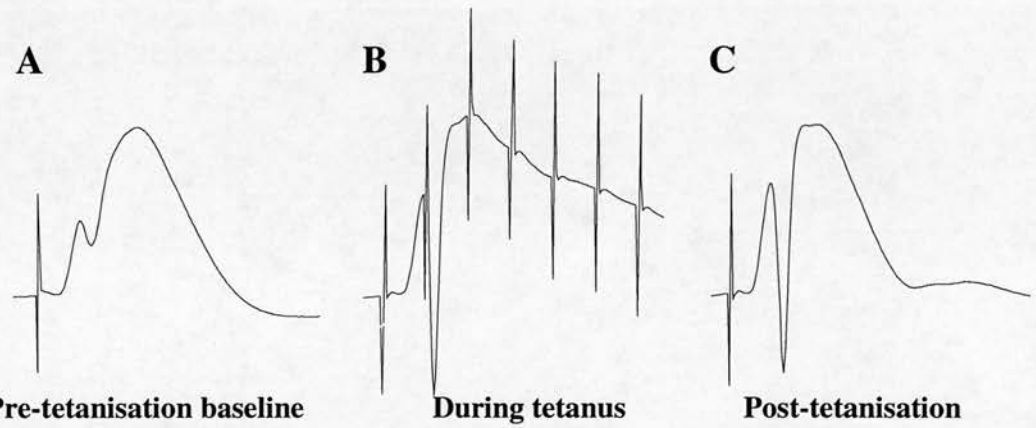


Figure 2.3 Effect of a train of high-frequency electrical stimuli on the size and shape of dentate gyrus potentials evoked by perforant path stimulation. (A) Example of a baseline response with the stimulus intensity adjusted so as to evoke a small population spike. (B) The shape of the waveform during a 400 Hz tetanus. Granule cells are unable to emit an action potential after every stimulus because of recurrent inhibition, hence the presence of only a single spike. The remainder of the waveform is broader than that seen after a single pulse. This is partly due to the contribution of the NMDA receptors to high but not low frequency evoked responses (see text for details). (C) Evoked response to the same intensity stimulus as in A, measured 30 min after tetanisation. Both the slope of the EPSP and the height of the population spike are increased, reflecting the greater synaptic efficacy which comprises long-term potentiation.

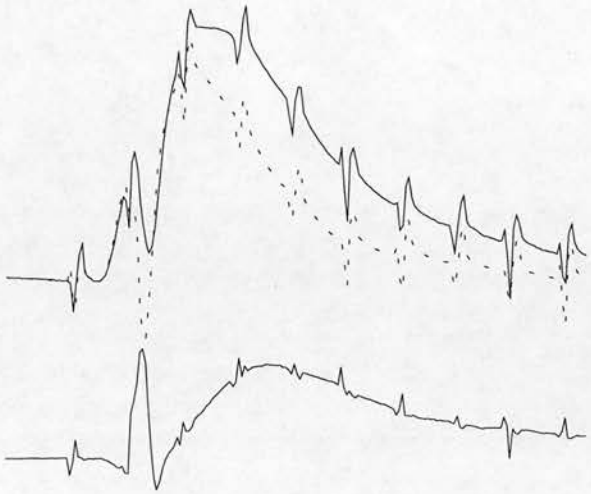


Figure 2.4 Contribution of NMDA receptor current to the evoked response during a tetanus. Top traces represent the waveform during a tetanus before (solid line) and 90 min after (dotted line) intra-peritoneal administration of the NMDA-receptor blocker CPP-ene. The lower trace was obtained by subtracting the post-drug from the pre-drug waveform, and represents the contribution of NMDA receptors during a normal tetanus. The initial spike at the beginning is the difference contributed by the induction of LTP.

The post-tetanic increase in EPSP size comes about because tetanisation induces a change in synaptic strength, so that a stimulus of the same strength as previously now results in a greater influx of ions. The increase in population spike is more complex and not fully understood. Part of the increase can be explained simply because the extra ion influx through the ion channels produces greater depolarisation at the cell soma and induces more granule cells to cross their thresholds for firing action potentials. However, the increase in spike height is proportionally much greater than the increase in EPSP size: in other words, if the post-tetanisation stimulus intensity is lowered until it evokes a field potential of the same size as before tetanisation, the population spike will still be considerably larger than previously. This residual spike potential which is unaccounted for by the EPSP is called E-S potentiation (Abraham and Goddard, 1984).

E-S potentiation can be illustrated more clearly if the spike height is plotted against the EPSP slope (an E-S curve) on logarithmic axes to linearise the curve (*e.g.* see Abraham and Bliss, 1985). The relationship before tetanisation is shown by the hollow circles in Fig. 2.5. After tetanisation, the curve is seen to be shifted to the left, meaning that an EPSP of a given size evokes, after LTP induction, a bigger population spike than before (independent of the stimulus intensity used to elicit the response). This increase (or "left-shift"), shown by the filled circles in Fig. 2.5, is E-S potentiation, the cause of which is unknown at present.

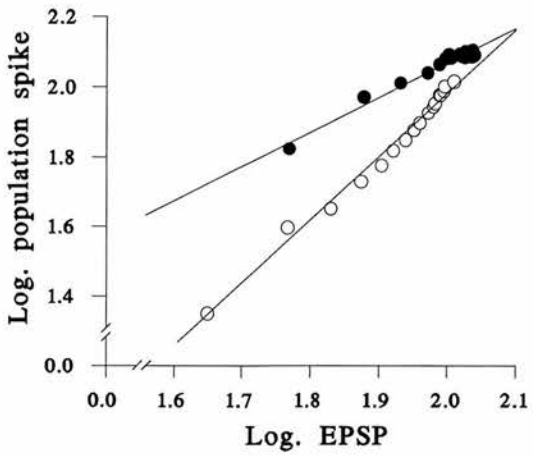


Figure 2.5 Log/log plot of EPSP versus population spike before (hollow circles) and after (filled circles) induction of LTP. Note that after tetanisation, an EPSP of a given size evokes a larger population spike than previously, reflecting the increased granule cell excitability which comprises E-S potentiation. However, increases in the EPSP produce a smaller proportional increase in the population spike, as evidenced by the decreased slope of the linear regression line after LTP.

Another feature of the post-tetanisation E-S relationship also becomes apparent: that of slope depression. This refers to the finding, also seen in Fig. 2.5, that in addition to being shifted to the left the E-S relationship has a smaller gradient so that although the spike at low current intensities is bigger than would be predicted on the basis of the pre-tetanisation EPSP size, at higher intensities this difference is less marked. The cause of slope depression was investigated in some detail by Kairiss *et al.* (1987) who suggested that because the depression was eradicated by inhibition blockade with the GABA antagonist picrotoxin it was therefore probably due to changes in inhibitory as well as excitatory synaptic strength. Recall that feedforward or feedback ("recurrent") inhibition only affects the population spike and not the EPSP because of its longer latency. If the inhibitory component of the spike response was potentiated after tetanisation then it would have produced a flattening of the E-S relationship, because increasing the size of the EPSP by increasing the current during IO curve measurement would recruit proportionally more inhibitory inputs, affecting the spike but not the EPSP.

2.3 Surgery

For the experiments to follow, standard procedures were used to implant electrodes bilaterally in the perforant path and dentate gyrus as follows: each rat was premedicated with midazolam (0.5 mg *i.p.*), anaesthetised with tribromoethanol (0.29 g/kg *i.p.*) and the scalp shaved. The rat was positioned in a stereotaxic frame (Kopf) with the tooth bar adjusted so that bregma and lambda lay on a horizontal plane. A midline scalp incision was made, the skin retracted and periosteum scraped from the skull surface with a scalpel blade. Burr holes 1 mm in diameter were drilled bilaterally at co-ordinates 3.8 mm posterior, 2.0 mm lateral to bregma, and 7.5 mm posterior, 4.0 mm lateral to bregma for

the recording and stimulating electrodes respectively. Two additional smaller holes made in the each of the frontal, parietal and occipital bones allowed eight jeweller's screws to be fixed to the skull to anchor the assembly. The frontal and occipital screws were attached by wires to gold pins, to provide ground and/or stimulus return for the electrophysiological recording.

The dentate gyrus (recording) electrodes were 75 μm monopolar Teflon-coated stainless steel wires wire-wrapped onto gold pins (Augat) and secured by means of either silver electrical paint coated with nail lacquer or solder. Stimulating electrodes were constructed in a similar manner except that the wires were a twisted pair with the tips separated vertically by 0.5-1.0 mm (Experiments 1 and 3) or monopolar with 0.5-1.0 mm insulation scraped from the tip (Experiments 2 and 4). Each electrode was positioned in the centre of a burr hole and lowered to a depth of 3.0 mm (recording) or 2.5 mm (stimulating) while test pulses of 700 μA intensity and 100 μs half-cycle duration were delivered to the stimulating electrode at 0.1 Hz. The final position of each recording electrode was adjusted to maximise the slope of the evoked potential, and of the stimulating electrode to maximise the height of the population spike.

After positioning, the electrodes were cemented in place with dental acrylic and the pins plugged into a plastic connector which was also cemented to the skull. The implant and wound edges were dusted with aureomycin antibiotic powder and the rats given an intra-muscular injection of buprenorphine (45 μg) for post-operative analgesia. For the next 5 days aureomycin was administered orally in the drinking water. The rats were allowed at least 2 weeks to recover from surgery before the experimental phase began.

2.4 Recording procedure

Electrophysiological recording began 2-6 weeks after surgery. Each rat in turn was carried in its home cage from the animal housing room to the recording room, where it was removed from the cage, placed in the recording chamber and connected to the recording and stimulating equipment via long flexible lightweight wires terminating in a plastic connector which plugged into the headcap. At the same time an unoperated control rat was placed in an adjacent, identical box to match as closely as possible the amount of handling and spatial experience for the operated and unoperated rats.

The general recording procedure for the experimental rats was as follows: each rat was acclimatised to the box for at least 20 min before any stimulation began. On the first day of testing the stimulus intensity was adjusted for each hemisphere so as to evoke a population spike of 1-3 mV in height and it remained at this intensity throughout the experiment except for IO curve measurement in Experiment 4, and for tetanisation. Evoked waveforms were sampled from each hemisphere consecutively at 0.1 Hz with

monophasic or biphasic square-wave stimuli of 100 μs half-cycle duration supplied by a constant-current stimulus isolator. The signal was amplified 200 times by a Grass amplifier, filtered (1 Hz-10 kHz) and fed via an A-D converter (Wild Vision) to an Archimedes computer (Acorn) for analysis and storage. Rats were recorded in the same order each day, preferably at the same time of day, and the average of 10 collected waveforms was used as the representative daily mean value.

Implanted rats with stable potentials in both hemispheres were assigned to the high-frequency groups, destined to receive tetanisation. Rats in which the evoked potential had deteriorated on one side during post-operative recovery because of presumed shift of the recording electrode were assigned to a low-frequency control group where appropriate, provided the stimulating electrodes on both sides were well placed (able to evoke a population spike). In this way they were able to serve as controls for the effects of implantation and brain stimulation.

2.4.1 Waveform analysis

Analysis of the collected waveforms either on-line or off-line consisted of measurement of the slope of the rising phase of the EPSP and the height of the population spike. The EPSP slope was measured using linear regression, between two fixed time points adjusted for each animal to be situated close to the onset of the positive deflection (Fig. 2.1). The height of the population spike was measured from the minimum of the spike to a line drawn between the two local maxima of the field-potential. The 10 waveforms sampled on each day from each hemisphere were averaged. Values for the EPSP were expressed as a percentage of the mean of the baseline days (including the pre-tetanisation value on the first LTP day where this was recorded). Because the size of the baseline population spike was adjusted to similar levels for all rats, and because proportional changes in this measure are generally very large and hence sensitive to small variations in the baseline, values for the population spike were expressed as the absolute deviation, in mV, from the baseline. Both EPSP and population spike were averaged across the two hemispheres.

2.4.2 IO curve recording and analysis

IO curves were measured daily throughout the baseline and tetanisation phases of Experiment 4. An IO curve consisted of a series of 0.1 Hz pulses increasing in intensity by 50 μA after every alternate pulse. Each curve consisted of 40 stimuli, ranging in intensity from 50 to 1000 μA.

For evaluation of within-animal IO curve potentiation, raw values were used. The two responses collected at each stimulus intensity were averaged for each rat across both hemispheres, and then averaged again across the 5 baseline days. Post-tetanisation curves were compared to the pre-tetanisation baseline average by expressing the recording at

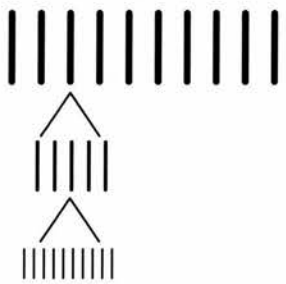
each stimulus intensity as the percentage increase over its corresponding baseline value. For between-animal comparison of pre- and post-tetanisation curves, the curves were first normalised as follows: curves from each hemisphere over the 5 baseline days were averaged. The last 6 pulses (from stimulus intensities 900 to 1000 μA) from each daily curve were averaged across the 5 baseline days to determine the baseline maximum of the averaged curve, which was set at 100%. For all curves, each point was then expressed as a percentage of this value.

2.4.3 Tetanisation and low-frequency control stimulation

During tetanisation, stimulus pulses set to the baseline intensity were increased in duration to 250 μs half-cycle and given as 400-Hz 10-pulse trains. In the low-frequency control stimulation condition pulses of the same duration and intensity were used. Each hemisphere was stimulated consecutively. Three types of tetanisation protocol were used to administer either 50 trains or 10 trains daily.

HF protocol 1

The high-frequency trains were administered in 10 bursts. Each burst consisted of 5 trains given at 1 s intervals and was followed by a 1 min pause, resulting in a total of 50 trains (500 single pulses) administered over a 10-min period. This protocol was repeated daily for 5 (Experiment 1) or 8 (Experiments 3 and 4) days.



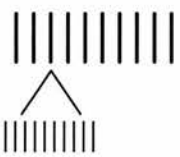
10 bursts at 1 min intervals

(each burst composed of)
5 trains at 1 s intervals

(each train composed of)
10 pulses at 400 Hz

HF protocol 2

The high-frequency trains were administered as 10 single trains given at 0.1 Hz (100 pulses). This protocol was repeated on each of 14 days.



10 trains at 1 min intervals

(each train composed of)
10 pulses at 400 Hz

HF protocol 3

The same as protocol 2 but trains were given at intervals of 1 min rather than 10 s. This protocol was given to rats in Experiment 3 after they had already received 8 days of 50-train stimulation.

The 2 low-frequency control protocols were as follows:

LF protocol 1

500 single pulses were given at 1 Hz.

LF protocol 2

10 single pulses were given at 0.1 Hz.

LF protocol 3

The same burst pattern was used as for HF protocol 1 except that each train was replaced by a single pulse.

2.5 Behavioural training

2.5.1 Spatial training

Apparatus

Spatial training was carried out in a watermaze as previously described (Morris, 1984). Briefly, the watermaze (Fig. 2.6) consisted of a white, featureless circular pool 2 m in diameter and 60 cm deep, filled to within 30 cm of the top with cloudy water warmed to 26 ± 1° C. The platform was 10 cm in diameter with a roughened white surface, and was submerged 1-2 cm below the water level. It was positioned in the centre of either the north-east or south-west quadrant of the pool (counterbalanced across groups) approximately 50 cm from the edge of the pool. Directly above the pool a concealed video camera recorded the path of the swimming rat and relayed the signal to an image analyser in the control room which converted the rat's position to *x*-*y* co-ordinates to be collected and analysed by a computer. Visual cues in the room included a wire rack hung with towels, white curtains gathered together at the edge of the pool, a tall chair, 4 floodlights, posters on the walls and 2 doors. In addition there were multiple olfactory and auditory cues from the computer and tracking equipment in an adjacent control room (where the experimenter remained during a trial), air conditioning vent, sink and rubbish bin. Care was taken to ensure that none of the cues could act as a direct signpost to the hidden platform, and to support this it has been repeatedly demonstrated that rats with lesions to the hippocampus cannot solve the task in this apparatus (*e.g.* Davis *et al.*, 1992).

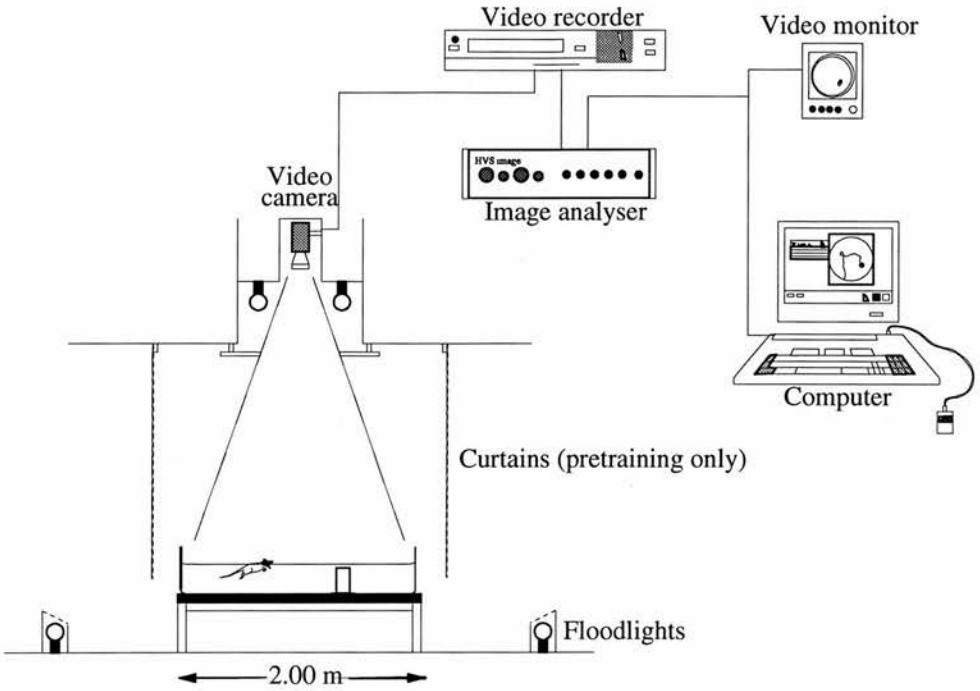


Figure 2.6 Diagram of the watermaze apparatus, courtesy of Caroline Stewart. The rat is placed in a 2 m diameter tank of water, in which is concealed a submerged platform. A video camera overhead records the path of the rat as it searches for the platform and relays it to an image analyser, which digitises the path and assigns each point an *x*-*y* co-ordinate. A computer then plots the path and calculates such parameters as the rat's speed and the time spent in each quadrant of the pool.

Procedure

For all trials in the pool, each rat was carried to the watermaze room in a covered cage. The cage was rotated backwards and forwards in a horizontal plane *en route* to try to disrupt dead reckoning on the basis of vestibular information, and thus encourage a spatial orientation based mainly on local room cues. Several days before spatial training took place rats were pretrained in the watermaze with the white curtains drawn around the pool to obscure the room cues. The position of the slightly submerged platform was indicated by the experimenter, and over 6 trials the rats became accustomed to swimming in the pool and learned to climb onto the platform. The platform was in a different place with respect to the room from the training phase, and was not moved between trials. Pretraining took place before the start of baseline recording.

Spatial training took place on the day after the final baseline recording day. The curtains were drawn back to reveal the room, and the rats were trained to swim from various starting positions to the platform, which was hidden in a fixed location with respect to the now visible room cues. Two spatial training protocols were used:

Protocol 1

A total of 8 trials were given at 2 h intervals. Two h after the final trial an absent-platform test was given.

Protocol 2

A total of 12 trials were given with alternating 30 s and 2 min intervals. Two min after the final trial an absent-platform test was given.

During each trial the latency of the rat to find the platform was recorded. If the platform was not found within 120 s the rat was placed on it by hand. At the end of each trial the rat remained on the platform for 30 s. The percentage of time spent searching in the appropriate quadrant of the pool during the absent-platform test was used as an index of spatial learning.

2.5.2 Conditioning

Training on the discrimination task began after the spatial training and tetanisation phase of Experiment 4. On the day that baseline electrophysiological recordings began, food deprivation was started and continued until the rats reached 80% of their initial body weight, from which point onwards they received enough food daily to maintain that weight throughout the remainder of the experiment. Pretraining for the conditioning experiment began after the 5th tetanisation session and lasted for 3 days. During this time daily tetanisation was continued. Conditioning proper started after the 8th and final tetanisation session.

Apparatus

The apparatus consisted of two identical aluminium Skinner boxes (Campden Instruments, Ltd.) with grid floors. During all training sessions the rats were run in pairs consisting, where possible, of an experimental animal in one box and a control in the other. Lighting was provided by a jewel light in the centre of the ceiling and the sound which served as the conditioned stimulus (CS) was emitted from a pair of speakers above the ceiling of each box. Two types of sound were produced: a 4000 Hz tone or a 20 Hz train of clicks. Both sounds were adjusted to an intensity of 82 dB and lasted 10 s. A food hopper guarded by a Perspex flap was set into one of the walls adjacent to the Perspex inner door. When a rat pushed its nose into the hopper the movement of the flap actuated a microswitch and was registered by the computer as a response. Food pellets (Noyes) were delivered singly to the hopper.

Procedure

A rat was placed in a Skinner box (the same box for each rat on each day, counter-balanced across the groups) and allowed to acclimatise for 10 min, after which 24 food pellets were delivered to the hopper on a variable-time 60 s schedule. On the first day the flap was fixed in a raised position allowing free access to the hopper, and on the next 2

days the flap was in its normal position and the rat was required to push the flap up to obtain the food.

Each conditioning session consisted of 8 trials, 4 of which were presentations of the CS+ (click) and 4 of the CS- (tone), pseudorandomly interspersed with an intertrial interval of 5 min. The onset of the first trial was 5 min after the rat was placed in the Skinner box. Discrimination of the stimuli was measured as the elevation ratio, this being calculated as the response rate during the CS+ divided by the sum of the rate during the CS+ and the CS-.

2.6 Histology

After the completion of some experiments, rats were deeply anaesthetised with sodium pentobarbitone (100 mg *i.p.*) and perfused intracardially with 10% formalin, and the brains removed and stored in formalin for later sectioning. Sections (40 μm thick) were mounted and stained with cresyl violet to allow visualisation of the electrode placements.

2.7 Statistics

Statistical tests used to analyse the data were factorial or repeated-measures analysis of variance (F), one- or two-tailed paired Student's *t*-test (*t*) and Pearson product-moment correlation (*r*). Confidence limits were set to $p < 0.05$ throughout.

PART I

LTP saturation and spatial learning

Chapter 3 – Experiments 1 and 2

3.1 Introduction

At the time that the present study began, some of the most important findings in support of the plasticity/learning hypothesis came from the occlusion experiments of McNaughton *et al.* (1986) and Castro *et al.* (1989), where perforant path tetanisation was found to impair subsequent spatial learning. The power of the occlusion effect arises from the fact that tetanisation can be directed at a specific pathway. Because LTP "saturation" is thought to affect only the propensity for further synaptic weight changes without disrupting normal physiological functions such as synaptic transmission, the finding of a subsequent learning impairment can be attributed with greater confidence to the synaptic weight changes alone, since as far as is known these changes form the only connection between tetanisation and learning (Fig. 1.7).

Confidence in interpretation of the occlusion studies, however, relies on these two assumptions: that is, that tetanisation affects neither pathways other than those targeted by the stimulation nor functions other than plasticity. Before accepting that these findings truly demonstrate that spatial learning is dependent upon the freedom of perforant path synapses to change their strengths, it is necessary to ascertain whether the assumptions are correct. One of the motivations behind the experiments in the present study, therefore, was to determine whether an LTP-associated spatial learning impairment could still be elicited when some of its other possible causes were eliminated. The second motivation of these experiments was to use the occlusion effect (once validated) as a tool for probing the contribution of intrahippocampal pathways to spatial learning. Each of these reasons is considered below.

3.1.1 Alternative explanations for the "saturation effect"

Discussion of alternative explanations for LTP-associated learning impairment will focus on the second of the two occlusion studies (Castro *et al.,* 1989), because that experiment used the same apparatus as in the present study, and because the task is known to be hippocampally dependent (*e.g.* Morris *et al.,* 1982). The design of their experiment was as follows: rats were implanted bilaterally with perforant path stimulating electrodes and dentate gyrus recording electrodes. After recovery, they underwent 5 days of baseline electrophysiological recording followed by 14 consecutive days of high frequency stimulation. "Immediately" after the last tetanisation session the rats were trained on a watermaze task, using alternating 30 s and 2 min intervals. Twelve such trials were given

followed by an absent-platform test 2 min later. It was found that the 4 rats given high frequency stimulation did not learn the task, as measured either by acquisition (latency to find the platform) or by specificity of search on the absent-platform test. The 4 rats given low frequency control stimulation showed a marked improvement over the trials and spent significantly longer searching the training quadrant on the absent-platform test. This result strongly suggests that the application of high frequency stimulation had impaired the subsequent ability of the animals to learn the watermaze task. One possible reason is that perforant path synapses had been driven to their maximum sustainable strengths, thus precluding any further naturally occurring changes and blocking subsequent learning. However, certain features of the experimental design suggest alternative explanations for the impairment which are not dependent on putative effects on naturally occurring LTP. There are three main possibilities:

(1) Insufficient anatomical specificity of tetanisation,

(2) Short-lasting effects of high frequency stimulation, or

(3) Electrolytic perforant path lesions

Insufficient anatomical specificity of tetanisation

In the Castro *et al.* study, a monopolar stimulating electrode was situated in the perforant path and the current return was provided by a screw located in the occipital bone, overlying the cerebellum. This practice allows for current spread across a wide region of brain tissue, between the electrode and the skull screw. One of the possible explanations for their findings, therefore, is that extra-hippocampal pathways were being disrupted by the high frequency stimulation. For example, tetanisation may have activated areas such as the vestibular system which are responsible for sensory processing needed to perform the task but probably unrelated to learning *per se.*

Short-lasting effects of high frequency stimulation

Rats in the Castro *et al.* study were given watermaze training very shortly after completion of tetanisation. The exact interval was not stated explicitly but the impression given was that it was of the order of minutes. Assuming a mean of 30 s swim time on each trial, the 12 training trials would have been completed by about 30 min after the last high frequency session. The possibility exists that short-term anterograde disruption of performance might disrupt learning independently of saturation-related effects (Collier *et al.,* 1982, 1987). It is not possible to ascertain whether short-lasting physiological abnormalities such as depression of the evoked perforant path response occurred in the Castro *et al.* study because they did not measure post-tetanisation evoked responses until 24 h following each tetanisation session (immediately prior to the next session on each

day). It may be, therefore, that seizures occurred following tetanisation and that a post-ictal confusional state was responsible for the learning impairment. Arguing against this is the fact that Castro *et al.* never observed afterdischarges in the immediate post-tetanisation phase of recording.

Electrolytic perforant path lesions

A final explanation for the post-tetanisation learning impairment is that administration of high frequency trains every day for 14 days resulted in progressive damage to perforant path fibres, which culminated in a lesion-induced learning impairment unrelated to synaptic plasticity. It is known that transection of the perforant path results in impairment on the watermaze task (Skelton and McNamara, 1992). Castro *et al.* controlled for this possibility by including in their experiment a third group of animals which received the same tetanisation regime as the experimental group, but which were not tested in the watermaze until 15 days later. If the spatial learning impairment had resulted from a tetanisation-associated lesion to the perforant path then these animals should also have been impaired on the watermaze task. However, they performed normally, suggesting that the effect of tetanisation on learning had dissipated during the 15 days of low frequency test pulses. This finding suggests that a permanent lesion is unlikely to have resulted from the tetanisation, or at least not one sufficiently severe to produce behavioural effects. However, an alternative but related possibility is that tetanisation produced a short-lived functional lesion of the perforant path which disrupted normal information processing rather than learning.

3.1.2 Occlusion as a tool for studying spatial learning

Because neither the mechanisms of spatial learning nor the functions of the hippocampal circuitry are presently known, a method for targeting plasticity in selected pathways would be of great use in dissecting out some of the components of spatial learning and their anatomical substrates. For example, if it were to be found that blocking plasticity in the perforant-path/dentate synapse impaired the learning of a new goal location but not navigation to an already-familiar place, then it could be hypothesised (a) that spatial learning and memory are anatomically separable, and (b) that spatial information is stored elsewhere but the locus of the initial learning is probably these synapses. Alternatively, if such LTP saturation affected (or did not affect) CA1 place fields, two synapses further along the trisynaptic circuit, then a hypothesis could be formed about the origin and role of place fields in spatial learning and navigation. Thus, the LTP saturation finding offers a means to produce what are effectively extremely localised lesions of a selective neural function, and therefore provides an additional motive for attempting to replicate and refine the occlusion effect.

3.1.3 Design of Experiment 1

The experiments in the present study were designed with the original intention of circumventing some of the objections to the Castro *et al.* result by attempting to narrow the anatomical locus of the spatial learning impairment to the perforant-path/dentate synapse. One means of accomplishing this is to make use of the cooperativity requirement for LTP induction by activating a separate but convergent pathway which terminates on the same set of postsynaptic granule cells. Using convergent stimulation it is possible to control the postsynaptic side of the process to modulate LTP induction and thus dissociate it from presynaptic activity. Because many of the postulated alternative reasons for the occlusion effect centre on non-specific results of presynaptic activity, this dissociation allows examination of the effects of holding LTP constant while varying presynaptic activity, or of holding presynaptic stimulation constant while varying (preferably blocking) LTP. In this manner it could be determined which factors are linked to the learning impairment.

Given a stimulus applied to the perforant path, there are two types of convergent input possible: inhibitory, from commissural inputs, and excitatory, from the contralateral dentate or from the other of the two components of the perforant path. This provides the opportunity for two types of experiment: one in which perforant path stimulation is maintained at a constant strength while LTP is blocked, and one in which perforant path stimulation is reduced below the threshold for LTP induction, while LTP is enabled by concurrent tetanisation of the convergent excitatory input. For the purposes of testing the occlusion hypothesis, the former technique is preferable because the latter still involves presynaptic tetanisation and it may be that dose-related effects of tetanisation, if there are any at all, are too subtle to be detected. The following experiment therefore suggests itself: it is known from the Castro *et al.* result that uncomplicated perforant path tetanisation results in impairment of spatial learning. If the most obvious consequence of this tetanisation, namely LTP induction, were to be blocked by concurrent commissural tetanisation then the occlusion hypothesis would predict a restoration of spatial learning. Because the convergence of the commissural influence is onto the postsynaptic cells, this finding would strongly suggest that non-specific extrahippocampal effects would be an unlikely cause of the learning impairment. Furthermore, because the blockade of LTP involves further brain stimulation it becomes unlikely that non-specific *intra*hippocampal effects could explain the results either, leaving only granule cell depolarisation and/or LTP induction as likely candidates for the impairment.

In order to conduct such an experiment the first step would be to replicate the original findings of Castro *et al.* This was the purpose of the first experiment to be described in this thesis. As will be seen, further testing of the occlusion hypothesis was precluded by

the unexpected finding that perforant path tetanisation resulted in no change at all in the ability of animals to learn a watermaze task.

Some changes were made to the Castro *et al.* protocol as follows. First, tetanisation intensity was increased and the duration (number of days over which tetanisation was administered) was reduced. In the McNaughton *et al.* (1986) circular platform study a spatial learning impairment was seen after only 2 consecutive days of tetanisation, and yet Castro *et al.* used the considerably more arduous protocol of tetanising daily for 14 days. The reason for this was never stated but a possible explanation is that the investigators felt that unless saturation of LTP was attained, there was a risk that a spatial learning impairment might be missed. Saturation was defined as a cessation of any further increases in evoked potential size, *i.e.* LTP induction to asymptotic levels. It has previously been found that a concentration of tetanic trains on a single day produces as much LTP as the same stimulation spread over several days (Jeffery *et al.,* 1990), with an apparent asymptote reached after only 2 days. Thus, to shorten the duration of the tetanisation phase of the experiment a more concentrated regime was given (50 trains daily instead of 10) for a shorter period of time (5 days instead of 14). In addition, because the grouping of trains into short bursts appears to produce better LTP than the same number of trains spaced evenly (Dragunow *et al.,* 1989, Jeffery *et al.,* 1990), a burst protocol was used here.

Second, in light of the findings on the circular platform task that anterograde learning effects appear to last for several days (McNaughton *et al.,* 1986), and because of the possibility (discussed above) that perforant path tetanisation might be accompanied by non-specific anterograde effects, it was felt prudent in this experiment to extend both the time after tetanisation and the inter-trial interval (ITI). Thus, after the final tetanisation session a 2-4 h delay was introduced before training began, and trials were separated by intervals of 2-4 h.

Third, in order to focus stimulation more discretely onto the perforant path, bipolar stimulating electrodes were used, the tips of which were separated by approximately 0.5 mm. Because of the close proximity of the stimulating current and its return, the chances of current spread outside the perforant path were minimised by this approach.

3.1.4 Pilot study

The first attempt to produce an LTP-associated learning impairment involved 3 rats implanted with perforant path and dentate gyrus electrodes as described in the General Methods section (Chapter 2). Two rats received bilateral tetanisation to the perforant path and the third received low frequency stimulation. The behavioural task was a reversal of the standard watermaze task: that is, rats were trained to locate a hidden platform prior to

any electrophysiological manipulations and then required to learn a new platform position after LTP induction. This procedure is analogous to the McNaughton *et al.* (1986) circular platform task, in which rats learned the location of the escape tunnel prior to LTP induction and were then tested on their ability to learn the new position afterwards.

The training procedure entailed 4 trials daily with an ITI of 30 s, over a period of 6 days, during which baseline electrophysiological recordings were conducted. The experimental rats then received 5 days of tetanisation using HF protocol 1, a procedure which appeared to have produced asymptotic LTP. After LTP induction the platform was shifted to the diagonally opposite location in the pool and the rats given an absent-platform test to assess recall of the previous platform position. They then received 10 training trials distributed over the next 3 days at a 4 h ITI, with the platform in the new location, followed by a final absent-platform test. Both tetanised rats appeared to have no difficulty learning the new platform position, as evidenced by the decrease in their latency scores, although when the platform was removed one rat showed no quadrant search preference and the other spent more time in the quadrant where the platform had been.

The 4 rats in the Castro *et al.* study showed no evidence of learning during the training trials, unlike the two animals here. From these preliminary findings it was tentatively concluded that reversal learning in the watermaze may be easier than new learning, possibly because it requires modification of a pre-existing representation rather than construction of a new one. For this reason it was decided to attempt a larger experiment in which only new learning was tested.

In the main experiment the electrophysiological procedures remained unaltered but the behavioural protocol was changed. It was initially intended to administer the same number of training trials as in the Castro *et al.* study, although at longer intertrial intervals for the reasons described above. However, because rats in the first replication appeared to be learning the task well after only 8 trials, training was stopped here in order to avoid the risk of ceiling effects obliterating any differential learning which might be occurring between the two groups. Thus, the training protocol was 1 trial every 2 h to a total of 8 trials, followed by an absent-platform test 2 h after the last trial.

3.2 Experiment 1 Methods

The time course of this experiment is shown in Fig. 3.1.

HF	surgery + recovery	baseline 5 days	watermaze pretraining	tetanisation 5 days	watermaze training
LF	surgery + recovery	baseline 5 days	watermaze pretraining	low frequency stimulation 5 days	watermaze training
UC		handling 5 days	watermaze pretraining	handling 5 days	watermaze training

Figure 3.1 Design of Experiment 1.

3.2.1 Surgery

Twenty-nine rats were implanted bilaterally with electrodes in the perforant path and dentate gyrus, as described in the General Methods. Stimulating electrodes in this experiment were bipolar with tips separated vertically by 0.5-1.0 mm.

3.2.2 Electrophysiology

Rats were assigned either to a high-frequency (HF) group (n = 8) to receive LTP-inducing stimulation, or to a low-frequency control (LF) group (n = 7) as described in the General Methods. The recording protocol consisted of a 5-day baseline period followed by 5 days of daily stimulation. Tetanic stimulation was administered according to HF protocol 1 and low frequency stimulation according to LF protocol 1. A post-stimulation baseline was recorded 30 min after the last train on each day.

3.2.3 Behavioural training

Behavioural training was carried out according to spatial protocol 1: that is, 8 trials were given at 2 h intervals with an absent-platform test 2 h after the final trial.

3.2.4 Histology

Seven rats from the HF group underwent histological analysis as described in the General Methods.

3.3 Experiment 1 Results

3.3.1 Electrophysiology

Eight rats received tetanisation, 7 rats received low-frequency stimulation and 14 unoperated control rats received an equivalent amount of handling. Table 3.1 shows the mean values for EPSP and spike in the HF and LF groups, over the baseline period and the last three days of the LTP-induction phase.

	Stimulus intensity (μA)	Baseline EPSP (mV/ms)	Baseline spike (mV)	Final EPSP (mV/ms)	Final spike (mV)	Training quadrant time (%)
LF (n=7)	539	1.92	2.85	1.65	1.85	41.3
	(85)	(0.34)	(0.27)	(0.31)	(0.27)	(6.1)
HF (n=8)	588	2.88	2.64	3.80	6.02	41.2
	(59)	(0.55)	(0.35)	(0.81)	(1.21)	(5.0)

Table 3.1 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after stimulation and spatial performance (percentage time spent in training quadrant) for rats in low- and high-frequency groups.

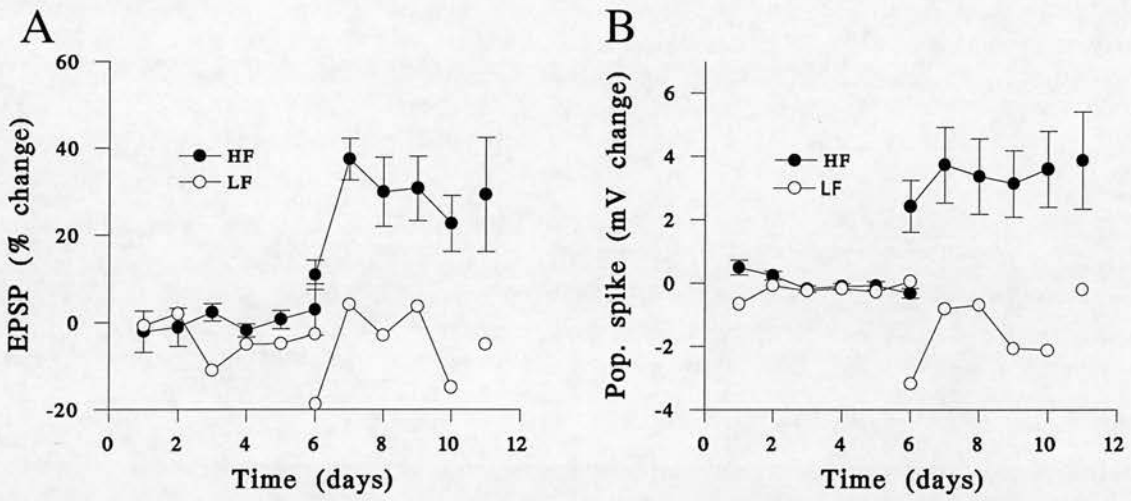


Figure 3.2 Normalised values for EPSP (A) and population spike (B) before, during and 24 h after 5 consecutive days of high frequency (HF) or low frequency (LF) stimulation. Post-stimulation values were recorded 30 min after the last train on days 6-10. Each point represents the group mean (±SEM) of 10 evoked waveforms. The LTP-induction phase commenced on day 6 and behavioural training began 2-4 h after the 5th stimulation session on day 10. The small decrease in EPSP and population spike recorded 30 min following 1 Hz stimulation each day had recovered completely by 24 h (day 11 time point).

The effects of the tetanic *vs.* low-frequency stimulation on the normalised values for EPSP and population spike are shown in Fig. 3.2A,B. Analysis of variance of the evoked potentials in the HF and LF groups during the baseline and LTP induction phases revealed a significant interaction between groups and phases reflecting the increase in the HF group, following tetanisation, in both the EPSP [$F(1,13) = 12.54$, $p < 0.01$] and the population spike [$F(1,13) = 12.41$, $p < 0.01$]. The values before and after 1 Hz stimulation were analysed further for the LF group using a one-tailed paired *t*-test. This revealed a significant decrease in the population spike ($t = 4.50$, $p = 0.002$) but not in the EPSP ($p > 0.1$). There was no change across the last 3 days of tetanisation in the LTP group for EPSP [$F(2, 14) = 1.20$, NS] or population spike [$F < 1$, NS], suggesting that both measures had reached a stable level, with increases of 30 % and 3-4 mV, respectively.

3.3.2 Behaviour

Fig. 3.3A shows the acquisition curve for rats in each group over the 8 watermaze trials. All groups showed a significant decrease in escape latency [$F(7, 182) = 14.01$, $p < 0.001$] with no difference between the groups [$F(2, 26) = 1.03$, NS]. Fig. 3.3B shows the performance of rats searching for the absent platform during the transfer test. All 3 groups spent significantly more time in the quadrant of the pool where the platform had been located [$F(3, 78) = 18.66$, $p < 0.001$]. There was no effect of LTP induction on time spent in the training quadrant [$F(2,26) < 1$, NS; Table 3.1].

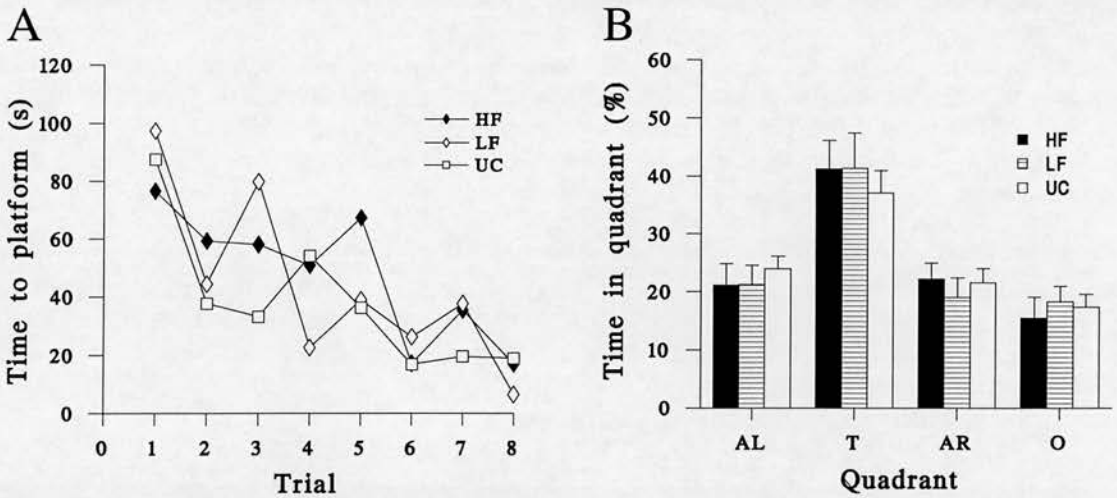


Figure 3.3 (A) Acquisition curves during water maze training for HF, LF and UC groups in Experiment 1. Data points represent the group mean latencies to find the hidden platform in each of the 8 trials. Inter-trial interval was 2 h. (B) Absent-platform test 2 h following the 8th trial. Values shown represent the group mean time (± SEM) spent in each of the 4 quadrants of the pool while searching for the platform. AL = adjacent left, T = training, AR = adjacent right, O = opposite.

3.3.3 Behaviour-LTP correlation

The behavioural and electrophysiological results from the HF group were analysed further. Pearson product-moment correlation revealed a highly significant correlation between the final level of EPSP LTP (as measured 30 min after the 5th tetanisation session), and the percentage of time spent in the training quadrant ($r = 0.95$, $p < 0.001$; Fig. 3.4A) A similar trend was seen for the population spike though this was not significant ($r = 0.68$, $p = 0.06$; Fig. 3.4B).

Rats from the LTP and LF groups combined were assigned on the basis of time spent in the training quadrant into good (n = 7) and poor (n = 8) learners (greater or less than the mean, respectively). Analyses of variance showed no significant relationship of learning with stimulus intensity, baseline EPSP or baseline population spike values [$F(1, 14) =$ 0.34, 1.58 and 0.27 respectively, NS; Table 3.2].

	Stimulus intensity (μA)	Baseline EPSP (mV/ms)	Baseline spike (mV)	Final EPSP (mV/ms)	Final spike (mV)	Training quadrant time (%)
poor learners (n = 4)	538 (103)	2.13 (0.11)	2.22 (0.57)	2.47 (0.14)	3.87 (0.80)	30.5 (2.7)
good learners (n = 4)	638 (63)	3.62 (1.00)	3.05 (0.36)	5.12 (1.36)	8.18 (1.77)	51.9 (5.5)

Table 3.2 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after stimulation and spatial performance (percentage time spent in training quadrant) for the tetanised rats from Experiment 1, divided into good and poor learners. Note the difference in the post-tetanisation EPSP and population spike size.

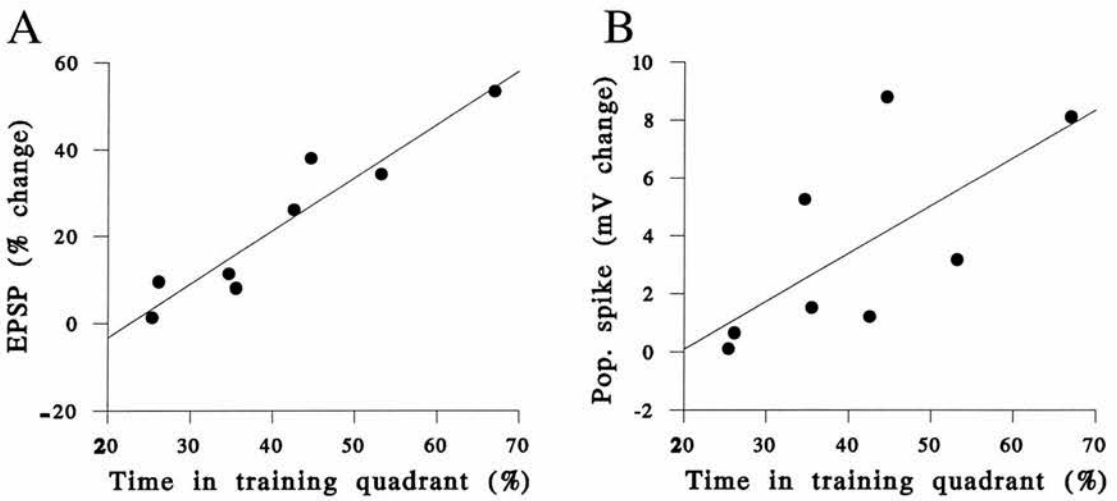


Figure 3.4 Linear correlation between spatial learning performance (percentage time spent in the training quadrant on the absent-platform test) and the cumulative level of LTP for the EPSP (A) and population spike (B).

3.3.4 Histology

All 7 brains showed stimulating electrode placements within the angular bundle bilaterally.

3.4 Experiment 1 Discussion

Two findings emerged from Experiment 1. First, there was no evidence of a spatial learning impairment following induction of LTP to apparently asymptotic levels. Second, there was a significant positive correlation between EPSP LTP and watermaze performance.

The first finding was somewhat surprising given the apparent reliability of the effects reported by McNaughton *et al.* (1986) and Castro *et al.* (1989). Obvious sources of the discrepancy from the Castro *et al.* study lie in the changes made to the watermaze and LTP induction protocols: that is, the number of days of tetanisation, tetanisation current

strength, the change in the electrode design and the timing and spacing of the watermaze trials. The second finding was even more surprising, given that the correlation between LTP and learning lay in the opposite direction to that which might have been predicted. That is, whereas it might have been expected that rats with the greatest amount of LTP would be the most impaired on the watermaze task, the exact reverse was found: these rats were the most accomplished learners.

With these findings in mind, a follow-up experiment was designed to address two questions: first, could the absence of a learning impairment in Experiment 1 have been due to alteration of the Castro *et al.* protocol, and second, was the LTP/learning correlation reliable? The original procedure of Castro *et al.* was therefore followed as closely as possible, with the exception that the tetanisation intensity was adjusted in the same manner as in Experiment 1. The reasons for the latter were threefold:

(1) Castro *et al.* never specified the current strength they used,

(2) a trial attempt at using a strong current, namely 1000 μA, produced visible behavioural effects (slight flinching) in a small number of rats during the first day of tetanisation, suggesting that a current this strong might risk inducing afterdischarges, and

(3) because a uniform current may produce different granule cell activation in different rats (because of variations in electrode placement), a possible correlation of LTP with learning ability such as that seen in Experiment 1 might be obscured by variable LTP induced by the varying postsynaptic activations involved.

Therefore, an attempt was made to standardise granule cell activation as before: that is, for the purposes of tetanisation a pulse of the same current needed to evoke a 1-3 mV population spike was increased in duration from 100 μs to 250 μs half-width.

3.5 Experiment 2 Methods

The time course of this experiment is shown in Fig. 3.5.

HF	surgery + recovery	baseline 5 days	watermaze pretraining	tetanisation 14 days	watermaze training	LTP decay
LF	surgery + recovery	baseline 5 days	watermaze pretraining	low frequency stimulation 14 days	watermaze training	LTP decay
UC		handling 5 days	watermaze pretraining	handling 14 days	watermaze training	LTP decay

Figure 3.5 Design of Experiment 2.

3.5.1 Surgery

Fifteen rats were implanted bilaterally with electrodes in the perforant path and dentate gyrus, as described in the General Methods. Stimulating electrodes in this experiment were monopolar with 0.5-1.0 mm insulation scraped from the tip, as for Castro *et al.* (1989).

3.5.2 Electrophysiology

Rats were assigned either to a high-frequency (HF) group (n = 8) to receive LTP-inducing stimulation, or to a low-frequency control (LF) group (n = 7) as described in the General Methods. The recording protocol consisted of a 5-day baseline period followed by 14 days of daily stimulation. Tetanic stimulation was administered according to HF protocol 2 and low frequency stimulation according to LF protocol 2. For the first tetanisation day, rats in the first replication received tetanus pulses at 1000 μA intensity. However, two rats flinched during the trains, suggesting that the current intensity was too high and either spreading outside the hippocampus or producing perforant path activation that was detectable to the animals. Henceforth the current used was the same as in the previous experiment: that is, of sufficient intensity to produce a 1-3 mV population spike when pulses of the baseline test duration (100 μs) were used, but with duration increased to 250 μs. EEG monitoring was conducted in all tetanised rats for the 30 min following stimulation to ensure that the stimulation was not provoking afterdischarges. A post-stimulation baseline was recorded 30 min after the last train on each day. After the tetanisation and training phase of the experiment was completed, evoked potentials from rats in the HF group continued to be recorded daily for 10 days and then on days 15, 20, 25 and 30 after tetanisation. Rats in the LF group received tetanisation according to the same protocol as was previously administered to those in the HF group, to determine whether an LTP/learning correlation could be observed if tetanisation followed training.

3.5.3 Behavioural training

Behavioural training was carried out as described in the General Methods section according to spatial protocol 2: that is, 12 trials alternating between 30 s and 2 min apart, beginning 10 min after the last train of the tetanisation series. An absent-platform test was given 2 min after the last training trial. After this, rats from the first replication (n = 11) then underwent 6 reinstatement trials in order to counteract extinction effects of the absent-platform test. Eleven days later they were given a second absent-platform test to ascertain retention of the platform location.

3.6 Experiment 2 Results

3.6.1 Electrophysiology

Evoked potentials from 2 rats in the LF group deteriorated over the course of the experiment and all data from these animals have been excluded. There were therefore 8 rats receiving tetanisation, 5 rats receiving low-frequency stimulation and 8 unoperated control rats receiving an equivalent amount of handling. There was no evidence of abnormal EEG activity in the tetanised rats during the immediate post-tetanisation period. Mean raw values for the EPSP and population spike are shown in Table 3.3, and normalised values over the 14 days of LTP induction are shown in Fig. 3.6A,B.

	Stimulus intensity (μA)	Baseline EPSP (mV/ms)	Baseline spike (mV)	Final EPSP (mV/ms)	Final spike (mV)	Training quadrant time (%)
LF (n = 5)	505	2.37	2.05	2.39	2.05	48.18
	(77)	(0.44)	(0.13)	(0.23)	(0.46)	(6.07)
HF (n = 8)	336	2.67	1.99	3.97	6.69	39.44
	(46)	(0.14)	(0.28)	(0.25)	(0.85)	(2.75)

Table 3.3 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after stimulation and spatial performance (percentage time spent in training quadrant) for rats in low- and high-frequency groups.

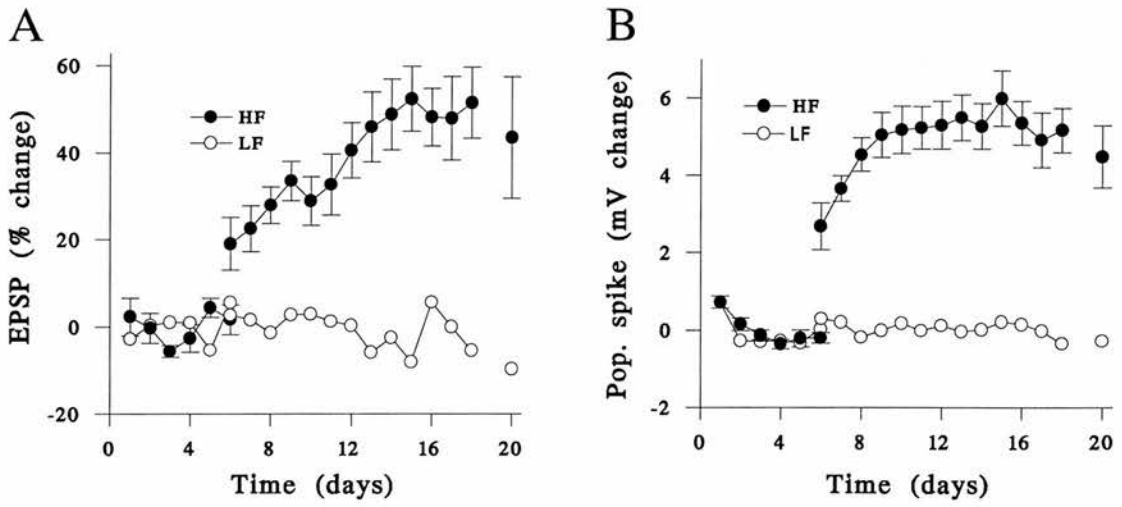


Figure 3.6 Normalised values for the EPSP and population spike (A and B) before, during and 24 h after 14 consecutive days of high-frequency (HF) or low-frequency (LF) stimulation. Each data point represents the group mean (± SEM) of 10 evoked waveforms. The LTP induction phase commenced on day 6 and behavioural training took place 10 min after the last stimulation session on day 19 (physiological recordings omitted).

The cumulative level of LTP in the HF group was higher than in Experiment 1, reaching a final increase of 50% for the EPSP and 5-6 mV for the spike, despite the rats having received a smaller total number of trains (140 *vs.* 250). Analysis of

variance of the evoked potentials during the baseline and LTP induction phases revealed a significant interaction for both the EPSP [$F(1, 11) = 23.31$, $p < 0.001$] and the population spike [$F(1, 11) = 48.43$, $p < 0.001$]. There was no change across the last 3 days of tetanisation in the LTP group for EPSP [$F(2, 14) = 1.02$, NS] or population spike [$F(2, 14) = 1.00$, NS], again suggesting that both measures had reached a stable level.

3.6.2 Behaviour

Acquisition curves for the watermaze training (Fig. 3.7A) revealed a significant decrease, over the 12 trials, in latency to find the platform [$F(11, 198) = 6.26$, $p < 0.001$], and all groups spent significantly more time in the training quadrant during the transfer test [$F(3, 54) = 47.89$,$P < 0.001$]. There was no significant difference between groups either for acquisition [$F(2, 18) = 2.21$, NS], or during the transfer test [$F(2, 18) = 1.76$, $p > 0.10$].

The slight decrease in time spent in the training quadrant shown by the HF group as compared with the LF and UC groups (Fig. 3.7B) was analysed further by considering only the first 30 s of the transfer test, but still failed to reach significance [$F(2, 18) = 1.79$, $p > 0.10$].

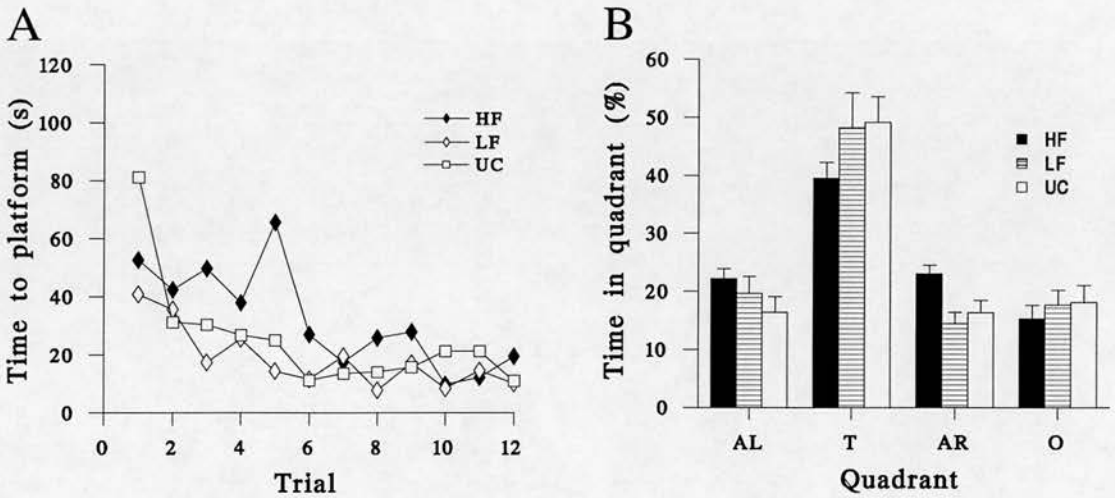


Figure 3.7 (A) Latency data for the 12 watermaze trials for the HF, LF and UC groups. Inter-trial interval alternated between 30 s and 2 min and trials began 10 min after the last HF session. (B) Absent-platform test 2 min after the last trial (group mean % quadrant time ± SEM). The HF group showed a slight trend towards spending less time in the training quadrant but this difference was not significant.

Rats from the first replication (n = 11) underwent a second absent-platform test 11 days later, to determine whether the decay rate of LTP correlated with retention of the watermaze task. However, the mean (± SEM) time spent in the quadrant where the platform had been was 25.19 (± 1.55) %, *i.e.* at chance levels, suggesting that after this length of time the rats either did not remember where the platform had been or did not

think it was likely still to be in the same place. Because a spread of retention scores could not be obtained, a correlation with LTP decay was not performed.

3.6.3 Behaviour-LTP correlation

LTP and LF rats were assigned on the basis of time spent in the training quadrant into good (n = 5) and poor (n = 8) learners (greater or less than the mean, respectively). One-way analyses of variance again showed no significant relationship of learning with stimulus intensity, baseline EPSP or baseline population spike values [$F(1, 12) = 0.37$, 0.32 and 0.33 respectively, NS; Table 3.4].

	Stimulus intensity (μA)	Baseline EPSP (mV/ms)	Baseline spike (mV)	Final EPSP (mV/ms)	Final spike (mV)	Training quadrant time (%)
poor learners (n = 5)	335 (71)	2.63 (0.21)	1.83 (0.44)	3.70 (0.32)	5.41 (0.94)	34.6 (1.59)
good learners (n = 3)	337 (54)	2.72 (0.20)	2.25 (0.18)	4.42 (0.29)	8.81 (0.47)	47.5 (3.19)

Table 3.4 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after stimulation and spatial performance (percentage time spent in training quadrant) for the tetanised rats from Experiment 2, divided into good and poor learners.

Pearson product-moment correlation again revealed a significant correlation between the final level of LTP of the EPSP, and the percentage of time spent in the training quadrant ($r = 0.80$, $p < 0.05$; Fig. 3.8). As in Experiment 1, a similar trend was seen for the population spike but this was not significant ($r = 0.53$, $p = 0.17$). Fig. 3.9 illustrates LTP acquisition for the good *vs.* poor rats over the 13 days of tetanisation.

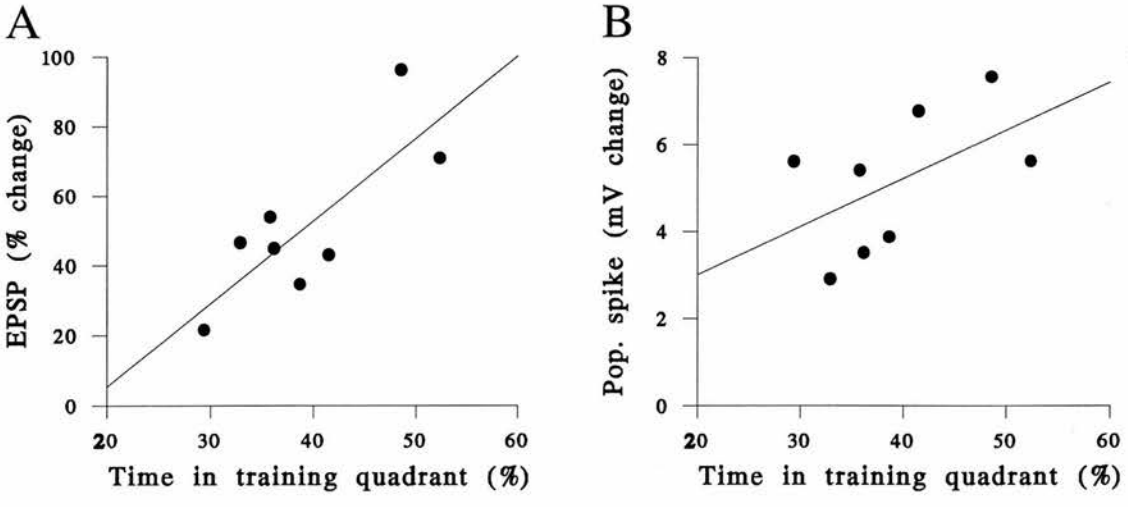


Figure 3.8 Linear correlation between spatial learning performance and cumulative LTP for the field potential (A) and population spike (B).

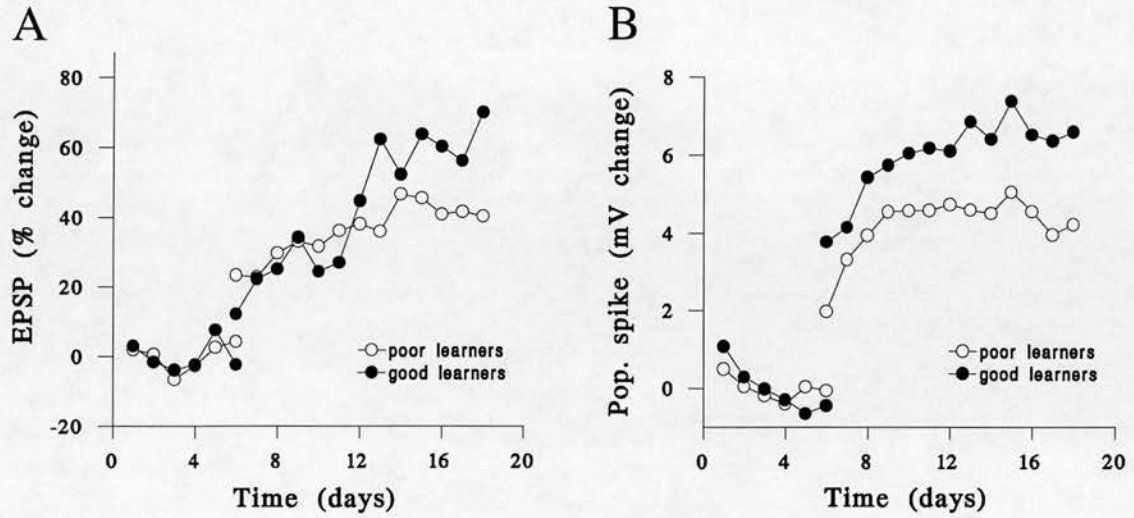


Figure 3.9 Comparison of the acquisition of LTP for good learners (n = 5) and poor learners (n = 3). LTP induction began on day 6. By the final day of tetanisation, LTP of the EPSP (A) appears to have stabilised at a low level in the poor learners but is still increasing in the good learners. The population spike appears to have stabilised in both groups (B), but at a higher level in good learners.

3.6.4 Decay of LTP

The exponential decay curves for the good and poor learners are shown in Fig. 3.10. Data from individual animals were too variable to allow individual curve fitting, given the small numbers involved. However, curves fitted to the group mean data yielded a decay constant (k) for the EPSP and population spike.

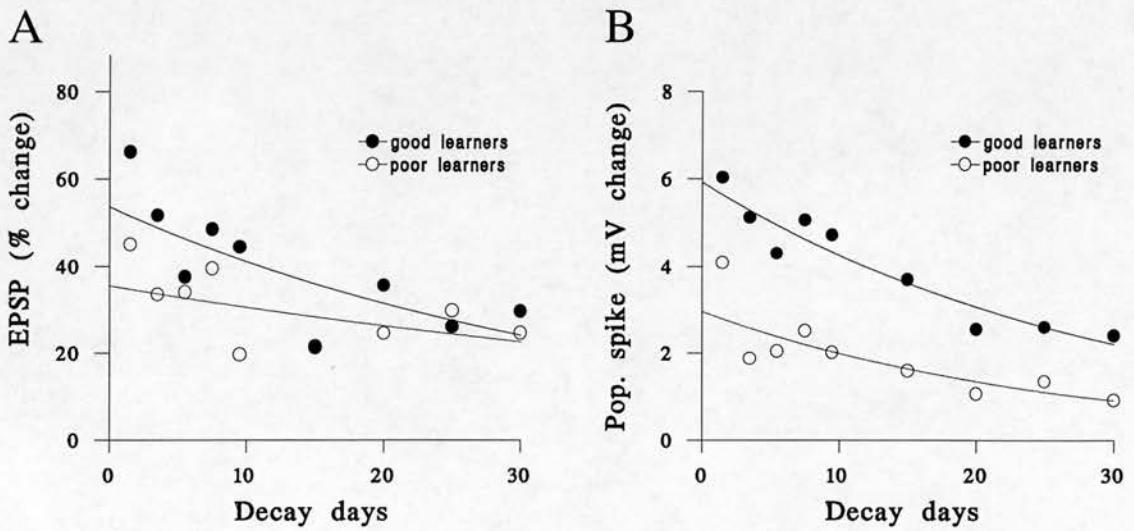


Figure 3.10 Comparison of LTP decay for good and poor learners for the EPSP (A) and population spike (B). The first 5 data points in each graph represent the mean of two days, the remainder are the values collected on a single day. The upper curves represent the exponential function for the good learners and the lower curves the function for the poor learners. The decay was slightly faster in good learners for the EPSP and in poor learners for the population spike. Population spike values remained significantly higher in the good learners throughout the decay period.

The values for the EPSP were k = -0.027 d^{-1} and k = -0.015 d^{-1} for the good and poor learners respectively. The values for the population spike were k = -0.033 d^{-1} and k = -0.039 d^{-1} for the good and poor learners respectively. Although it is not possible to compare these values statistically, the fact that the EPSP decayed slightly faster and the spike slightly slower for the good learners than the poor learners suggests that these parameters did not differ greatly between the two groups.

3.6.5 Tetanisation of the low-frequency controls

Finally, the 5 rats which had received low frequency stimulation as a control condition underwent tetanisation according to the same protocol (13 days of burst stimulation at 50 trains daily) and the final level of potentiation was correlated with the previous performance in the watermaze. One rat showed deterioration of the evoked response during this time. The correlation for the remaining 4 rats was positive for both EPSP and population spike (r = 0.22 and 0.42, respectively) but not significant.

3.7 Discussion of Experiments 1 and 2

Two results emerge from these two experiments. First, induction of LTP in the dentate gyrus to apparently asymptotic levels had no effect on the ability of rats to learn a watermaze task even when the Castro *et al.* (1989) protocol was followed nearly exactly. Second, in both experiments the cumulative level of LTP correlated highly and *positively* with performance on the task. These two findings will be considered in turn.

3.7.1 LTP "saturation"

In Experiments 1 and 2, no significant learning impairment was seen following induction of LTP to levels as high as those reported by Castro *et al.* (50% increase in EPSP in Experiment 2). There was a slight, non-significant trend towards a spatial learning impairment seen in the HF group in Experiment 2, as reflected both in the acquisition curve (Fig. 3.7A) and the absent-platform test (Fig. 3.7B). This may be because the induction of LTP had moved synapses sufficiently far towards saturation to produce the beginnings of a spatial learning deficit. It is also possible however that the effect was due to chance assignment of naturally poor learners to the HF group. In support of this interpretation, there is in this group (unlike Experiment 1) an uneven distribution of LTP levels with a bias towards the lower end of the range (Fig. 3.8). If synaptic plasticity is related to spatial learning (see below), it is possible that rats scoring low on both these measures were over-represented in this group by chance alone.

The absence of a spatial learning impairment after apparently maximal induction of LTP may have occurred for methodological or theoretical reasons. At the time that the

experiments in this thesis were being conducted, several other groups were also attempting to occlude spatial learning by means of LTP induction in the perforant path. The first study, by Robinson (1992), was published in *Hippocampus* shortly after the present Experiment 2 was completed. The remainder consisted of varying attempts to replicate the Castro *et al.* (1989) findings in the watermaze and were published, together with Experiments 1 and 2 of the present study, in *Hippocampus*, **3(2)** (1993). Because the analysis to be presented below applies equally well to the collection of studies as a whole, a brief synopsis of their findings is presented in the Appendix.

Some possible explanations for the discrepancy of Experiments 1 and 2 and of these 5 other studies from the original reports (McNaughton *et al.*, 1986, and Castro *et al.*, 1989) are as follows.

(1) Incomplete dorso-ventral potentiation,

(2) Incomplete potentiation across the perforant path,

(3) Incomplete saturation of LTP in potentiated synapses, or

(4) Mediation of spatial learning by some other mechanism.

Incomplete dorso-ventral potentiation

The first possibility concerns the extent to which the entire length of the hippocampus is activated by the stimulating positions used both in Experiments 1 and 2 and by others. Histological examination of the stimulating electrode tracks in a subset of rats showed the electrodes to be located in the angular bundle of the perforant path, which projects to a large part of the rostro-caudal extent of the dentate gyrus (Amaral and Witter, 1989). However the two components of the perforant path do not project equivalently to all parts of the hippocampus, the medial path contribution becoming progressively less in more ventral regions (Gall *et al.*, 1984). Thus, an electrode located principally in the medial perforant path might evoke a strong response in the dorsal hippocampus (DHC), from where the recordings were made, but a weaker response (and hence less LTP) in the ventral hippocampus (VHC). In support of this, data from immediate-early gene induction suggest that the LTP-associated increase in *zif/268* following tetanisation at comparable stimulating locations is more intense in the DHC than the VHC (Cole *et al.*, 1989; p. 474). An explanation therefore for the present failure to observe an LTP-related learning impairment may be that the ventral hippocampus was not activated enough during tetanisation to enable saturation of LTP, thus allowing a residual capacity for plastic change in the VHC to compensate for the loss of dorsal LTP. Arguing against this is the observation that while good spatial learning may be supported by the DHC in the absence of the VHC, the converse does not appear to be true, at least following aspiration lesions (Moser *et al.*, 1993). This suggests that the contribution of the VHC to spatial

learning may be small under normal circumstances. Whether the VHC is able to take over the spatial learning role of the DHC following fibre-sparing lesions to the latter, or following blockade of dorsal LTP, remains to be demonstrated.

The possibility that the stimulating electrode position used in Experiments 1 and 2 did not sufficiently activate more ventral regions of the hippocampus was investigated further in the present study. Twelve rats were anaesthetised and prepared for electrophysiological recording in the usual manner, as described in the General Methods. An attempt was then made to correlate stimulating electrode position with the evoked response profile from various recording and stimulating sites. Either the stimulating position was held constant at the usual site (bregma -7.5 mm A-P, 4.0 mm M-L) while the recording electrode was shifted by up to 1 mm in the M-L direction and up to 4 mm along the longitudinal axis of the hippocampus, or the recording electrode was held in the usual position (bregma -3.8 mm A-P, 2.0 mm M-L) while the stimulating electrode was shifted by up to 1 mm in all directions. Responses were recorded at various depths in 200 μm increments from the brain surface to 8 mm below. Evoked potentials were only seen in two animals at a depth greater than 4 mm below brain surface, and neither was associated with a population spike. Histological analysis revealed that the recording electrode routinely reached the ventral dentate gyrus during more posterior penetrations. The conclusion therefore is that failure of perforant path stimulation to activate the ventral hippocampus using conventional stimulation sites is a valid possibility.

Incomplete potentiation across the perforant path

The second possible reason for the preservation of apparently normal learning following LTP induction is that even if the stimulating electrode had been placed so as to spread current to fibres projecting to all parts of the hippocampus, not all synaptic terminals of these fibres necessarily achieved potentiation. This could occur either because some fibres were not activated sufficiently strongly to produce action potentials, or because some synapses will always resist potentiation even if fibre activation is 100%.

Because IO curves were not performed in Experiments 1 and 2, it is not known whether the stimulus pulses used during tetanisation would have been eliciting a maximal dentate response, although since the pulses were 2.5 times the duration needed to elicit a healthy population spike this seems likely. If the pulses were submaximal then the synaptic terminals of the residual unstimulated fibres would not have been potentiated (because of the associativity requirement, but see Bonhoeffer *et al.,* 1990). However, definitively supramaximal stimulus pulses were used in some of the other studies described in the Appendix (*e.g.* Robinson, 1992, Cain *et al.,* 1993). If the absence of a learning impairment in those studies was due to incomplete LTP saturation then this must have occurred because of the second reason: that even with 100% stimulation of the perforant

path fibres, some synapses managed to escape potentiation. This may happen either because an activated fibre does not have a 100% probability of releasing neurotransmitter, or because the other half of the associativity requirement was not met: that is, not all granule cells depolarised sufficiently to unblock their NMDA receptors during the tetanus. It may be that perforant path innervation density of the dentate gyrus is not uniform, so that some granule cells receive enough inputs that they will cross the firing threshold when all these inputs are activated together, whereas others are more sparsely connected and never cross threshold, even during a tetanus. This seems a rather unlikely explanation for incomplete potentiation. If even tetanic activation of all of the inputs of a granule cell is unable to drive it past its firing threshold then it is difficult to imagine what natural pattern of activation could do so: or conversely, what the function of a non-firing granule cell might be. An alternative possibility is that even a combination of presynaptic activity and postsynaptic depolarisation will not potentiate all synapses because LTP induction, like transmitter release, might be a probabilistic process. If some synapses escaped potentiation because of a probabilistic failure of either synaptic transmission or LTP induction then they would, in principle, still be potentiable: that is, they would still be available to mediate learning when necessary. Such a scenario might therefore explain why tetanised animals still appear to be able to learn a spatial task. However, in all of these studies tetanisation was repeated daily, sometimes for many days. If potentiation failure is due to chance, the only way in which a significant number of synapses could stay unpotentiated after repeated tetanisation to asymptote would be if each tetanus only potentiated a small number of synapses at a time, so that the rate of potentiation was approximately equal to the rate at which synapses were being restored to full plasticity by decay of their LTP. If this balance of potentiation and decay produced a stable level lying well below that which would be produced in conditions of full potentiation and zero decay, then the difference may be sufficient to support learning. This argument depends on the assumption that natural levels of stimulation are able to potentiate synapses which escaped tetanic potentiation: however, this seems rather unlikely, unless tetanisation partially blocks LTP induction in a way that natural activity does not. However, it will be shown in the next experiment that this last possibility is not without foundation.

Incomplete saturation of LTP in potentiated synapses

The third question relating to why tetanised animals in these experiments could still learn concerns how close the potentiated synapses were to their maximum levels, after tetanisation. That is, assuming that LTP in a single synapse is a graded phenomenon and not all-or-nothing, how "saturated" was the LTP in potentiated synapses? In Experiments 1 and 2 repeated tetanisation over many days resulted in a variable increase in the EPSP slope which stabilised, on average, at only 50% above baseline. Data from hippocampal

slices (*e.g.* Barrionuevo and Brown, 1983) show that under some conditions (such as in the presence of picrotoxin), nearly 200% increases in the EPSP are possible – considerably higher than the maximum found here or by Castro *et al.* (1989). The findings of different "maximal" LTP under different conditions raise the question of where the true limit to synaptic strengths might lie, and under what circumstances, if any, this limit may be reached *in vivo*. Furthermore, even if it was known how strong a synapse could theoretically become, given perfect LTP induction conditions, it is not known how closely the synapses must approach their maximum strengths in order for a learning deficit to ensue. The contribution of synaptic strength changes to learning depends both upon the magnitude of their change following a given input, and their lability (*i.e.* how readily they change in response to the input). Learning impairments could, in theory, be induced either by limiting the magnitude of weight change possible (for example by forcing synapses up towards a ceiling level, and thus restricting their dynamic range), or by decreasing their readiness to change (such as by blocking the NMDA receptor). There may be a functional difference in effects on learning between these two factors. Studies of LTP blockade with the NMDA-receptor antagonist AP5 suggest that synaptic plasticity need only be reduced by as little as 50% before a learning impairment becomes detectable (Davis *et al.,* 1992). However it is not known whether forcing synapses even to near-maximal strengths produces a similar reduction in their lability. It may be the case that, provided synapses are able to distribute their weights with relative ease, they can function within a narrow range of such weights. In addition, negative synaptic changes (long term depression) may supervene at higher LTP levels so as to enable sufficient information storage for the task to be learned.

Mediation of spatial learning by some other mechanism

The final explanation for the general inability of perforant path tetanisation to block spatial learning is that the results of the original experiments (McNaughton *et al.,* 1986, Castro *et al.,* 1989) were either spurious or had some other cause, and that even perfect tetanisation-induced blockade of synaptic plasticity, achieved by circumventing all of the pitfalls discussed above, would not block the learning of spatial tasks. There are two ways in which this could happen. The first is, of course, that perforant path synaptic plasticity does not mediate the learning of tasks like the watermaze. The second is that under *normal* conditions it mediates this type of learning but under conditions in which this mechanism is disabled, spatial learning occurs by a different process from the usual one.

Complete transection of the perforant path impairs performance in the watermaze (Skelton and McNamara, 1992), suggesting that the perforant path normally participates in this type of learning. However, it may be that either its synaptic plasticity is not used for this function, or it can be later compensated by some other mechanism. For example,

it may be that while LTP was indeed saturated in the dentate gyrus, LTP of the other entorhinal-hippocampal pathways was either partially or wholly spared, and able to take over the learning role of the dentate gyrus. Arguing against this possibility is the observation that lesions to the dentate gyrus profoundly impair watermaze learning (Sutherland and Rudy, 1988), suggesting that the remainder of the hippocampus is unable to compensate for complete loss of the processing contributed by this structure. However, it may be that loss of dentate plasticity while preserving the normal throughput of information is more easily compensated.

An alternative hypothesis is that information might be diverted elsewhere in these circumstances. Given that the information needed to solve the task is clearly *not* routed elsewhere when the perforant path or dentate gyrus is completely lesioned, any switching mechanism would have to operate selectively in conditions of impaired plasticity but not in conditions of complete blockade of information throughput. Transection of the perforant path produces retrograde degeneration of its source tissue, the entorhinal cortex (Skelton and McNamara, 1992) whereas as far as is known, blockade of plasticity affects only the relevant synapses. It may be that if incoming information meets up against a non-functional entorhinal cortex it will be poorly processed, whereas if it successfully proceeds as far as the perforant path/dentate synapses and fails to be processed because these synapses are non-plastic it can then be processed elsewhere, perhaps across one of the entorhinal-pyramidal synapses.

3.7.2 Correlation of LTP with behaviour

The second finding of Experiments 1 and 2 was that the magnitude of LTP induction correlated strongly with subsequent spatial learning ability. This finding was unexpected because the correlation lay in the opposite direction to that which would be predicted by the occlusion hypothesis. That is, rats showing the greatest levels of LTP should have been the most impaired at the spatial task, but paradoxically they were the best learners (Figs. 3.4 and 3.8). This trend became apparent from the first replication of Experiment 1, where the two tetanised rats far outperformed either the low-frequency or the unoperated controls, raising the possibility that LTP induction had somehow *facilitated* learning in these animals (*cf.* Berger, 1984). However, with further replications it became apparent that some control animals were able to learn as well as the first tetanised animals, and that some subsequent tetanised animals performed as poorly as the first controls. Thus, it seems likely that the relationship between LTP and learning seen in these experiments reflects a correlational but not necessarily a direct causal association between the two phenomena.

The finding of a correlation between cumulative LTP magnitude and learning provides circumstantial support for the plasticity/learning hypothesis, because both LTP and

learning are the end links in a chain of causes and effects of which synaptic plasticity is the shared factor (Fig. 1.7). If both learning and LTP resulted from synaptic efficacy changes then they would be expected to co-vary. However, the finding, as in these experiments, that they co-vary does not in turn prove that therefore they must both result from underlying synaptic changes. Some of the possible alternative explanations for the finding are explored in the next chapter.

Using the correlation to test the plasticity/learning hypothesis

Although the evidence contributed by the linear LTP/learning correlation is circumstantial, the finding of a quantitative relationship such as this opens up the possibility of designing interventional experiments which exploit this relationship to determine whether LTP and learning truly co-vary under all conditions, as they should if they are both dependent on synaptic plasticity. Interventional experiments, as mentioned earlier, possess more power than observational experiments because it is possible to gain control of just one link in a chain of putative causes and effects. For example, blockade of the NMDA receptor has been employed to test the plasticity/learning hypothesis (see Chapter 1) because it is known that this affects synaptic plasticity and hence blocks induction of LTP. Because in this scheme learning also depends on synaptic plasticity, modulation of this single factor (namely NMDA receptor availability) would also be predicted to affect spatial learning. However, the single observation that NMDA blockade reduces spatial learning would be undermined if the manipulation affected other, as-yet-undiscovered links in the scheme which connected the NMDA receptor and learning: for example, a possible role for NMDA receptors in the normal functioning of the visual cortex.

One way of more closely associating two phenomena like these is by means of a dose-response study, in which the factor which is thought to be the cause is modulated over a range of values. If the second factor is truly an effect of the first then it should vary in parallel. This tactic has been adopted in the NMDA/learning studies, where NMDA blockade was administered at low, medium, high or very high doses (Davis *et al.,* 1992) and spatial learning impairment found to vary proportionally. This approach strengthens the association but again, it does not rule out the possibility that other factors (such as visual impairment) also vary in parallel. A more sophisticated approach is enabled by the present finding of a within-animal correlation between LTP and learning. If this correlation arises because learning and LTP are both dependent on a common underlying mechanism, then modulation of synaptic plasticity should produce a covariation of LTP and learning, the slope of which *may be predicted on the basis of the known relationship.* In other words, not only is it possible to predict that increased NMDA blockade will produce an increased spatial learning impairment, but it should also be possible to predict

the exact *amount* of learning impairment which should accompany a given degree of receptor blockade (as determined by the reduction in induced LTP), using the LTP/learning relationship which is now known to exist.

This idea is explained by the diagram in Fig. 3.11. Suppose a dose of an NMDA receptor blocker is given which reduces synaptic efficacy changes to 70% of normal. According to the plasticity/learning hypothesis, it follows that LTP and learning should both be reduced too. If the linear LTP/learning correlation found in the present experiments exists because LTP and learning cannot be dissociated, due to a shared underlying mechanism, then the result should be that the LTP/learning data points after drug administration should still lie along the same line. The finding that the post-drug data points did not lie along a straight line would suggest that there was no simple transformation which could account for the change in LTP and learning: in other words, that there was probably some other factor involved, such as sensorimotor disturbance. Conversely, the finding of a preserved linear LTP/learning correlation after a drug treatment which affected synaptic plasticity would be strong evidence that this mechanism underlies both LTP and learning.

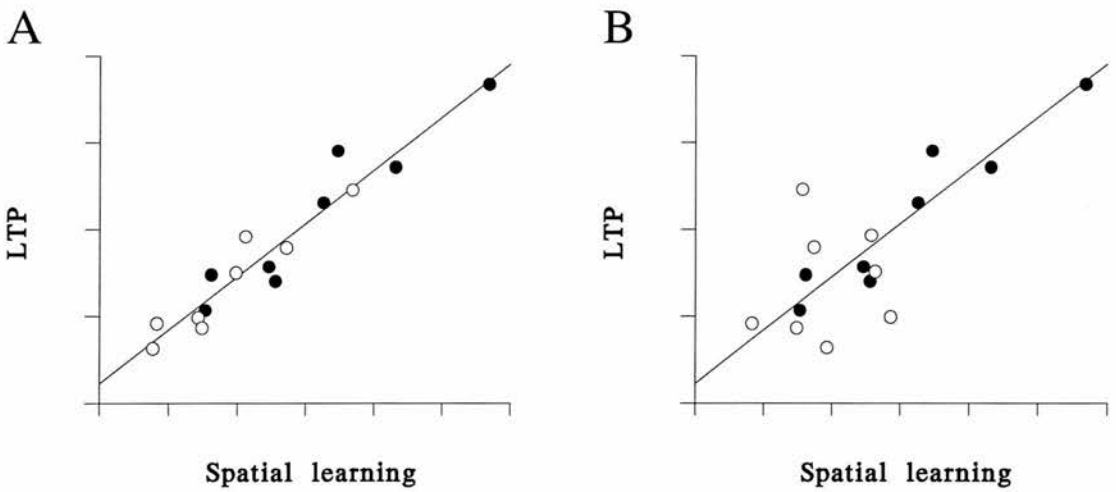


Figure 3.11 Relationship between LTP and spatial learning before (filled circles) compared with a hypothetical relationship which might exist after (hollow circles) administration of an NMDA receptor blocker which reduced LTP to 70%. The pre-drug data points were taken from Experiment 1. (A) If the LTP/learning correlation arises because of a common underlying mechanism, then after an intervention which modulates one factor, the other should vary along the same regression line. (B) If NMDA antagonists impair LTP and learning by different mechanisms then the relationship would be unlikely to remain linear.

3.8 Conclusion of Experiments 1 and 2

When the findings of Experiments 1 and 2 are considered together with those of the studies outlined in the Appendix, it appears that the LTP-related spatial learning impairment described by McNaughton *et al.* (1986) and Castro *et al.* (1989) is difficult or perhaps even impossible to replicate. As noted by the group responsible for the original studies (Korol *et al.*, 1993; see Appendix), this finding deprives the plasticity/learning

hypothesis of one of its "major lines of support". From the point of view of the present study, the question arises as to how best to proceed. There are two possible approaches: either an attempt could be made to discover the conditions under which the occlusion effect *could* be reliably obtained, or the experiments could be redesigned to pursue the plasticity/learning hypothesis by some other means. The second alternative was chosen here for the following reasons. First, if the results of the original two experiments were spurious, it is by no means certain that the occlusion effect ever could be obtained experimentally (assuming the plasticity/learning hypothesis is true) because of the methodological pitfalls discussed earlier. If the results of the two experiments were real and the subsequent failures-to-replicate due to methodological problems, then it is clear that the occlusion effect is, at the very least, somewhat fickle. This removes one of the major motivations of the present study to produce the effect: namely, to use it as a tool to explore spatial learning mechanisms. If the occlusion effect exists but is difficult to obtain then it is no longer useful for that purpose, and its sole value lies in the validation of the plasticity/learning hypothesis.

While the latter purpose is not a groundless reason for pursuing the occlusion effect, Experiments 1 and 2 produced a novel finding which suggests an alternative means of exploring the relationships between LTP, synaptic plasticity and learning. This finding is that repeated tetanisation resulted in a cumulative LTP level which correlated strongly with spatial learning ability. This effect has not been reported previously, and as explained above, it provides a potential means with which to quantify the relationship between LTP and learning. Part II of this thesis is therefore devoted to an exploration of this correlation.

PART II

LTP/learning correlation

Chapter 4 – Experiment 3

4.1 Introduction to Experiment 3

The finding in the previous two experiments that the cumulative level of LTP correlated with spatial learning ability raises several questions about the relationship between synaptic plasticity and learning. An investigation of some of these issues forms the basis of Part II of this thesis.

One possible explanation for the LTP/learning correlation is that the effectiveness of learning depends on the effectiveness of the underlying plasticity of the synapses, which is reflected in the magnitude of induction of LTP (Fig 1.7). There are several other possible explanations which need to be considered. One is that it is not LTP *per se* which co-varies with learning but rather an associated, pre-existing parameter, such as dentate gyrus excitability. For example, if there had been a correlation between the EPSP-spike relationship *prior* to tetanisation and a given rat's subsequent performance in the watermaze, this correlation could result in a secondary association of learning with LTP. Analysis of EPSP, population spike and stimulus intensity for good and poor learners, however, suggested that these parameters did not differ between the two groups. Nevertheless, comparison of the post-tetanisation shift of the whole IO curve with watermaze performance is warranted. This point will be returned to in the next chapter.

A second possible explanation for the LTP-learning correlation is that plasticity in the hippocampus may correlate with plasticity in other parts of the cortex, and that the spatial learning is really taking place elsewhere. Alternatively, both phenomena may correlate with non-specific brain states such as arousal. A more interesting possibility is that those rats which showed only low levels of LTP induction were those whose synapses were already approaching maximal strengths, either because they had already undergone considerable naturally occurring potentiation or because they had intrinsically lower ceiling levels of synaptic strength. Thus, the poor spatial learning performance may represent a true saturation-related effect. In support of this, comparison of LTP acquisition curves in Experiment 2 for good learners *vs.* poor learners shows that LTP in the poor learners appears already to have reached a plateau, whereas that in the good learners has yet to do so (Fig. 3.9). However, if this plateau represents true LTP saturation and is the explanation for the poor performance of these animals, the HF group as a whole would be expected to show an overall impairment in learning as compared with controls. This was not the case, although there was a non-significant decrease in time spent in the

training quadrant in the HF group in Experiment 2. This matter could be resolved conclusively by means of experiments in which spatial training takes place *before* LTP induction. If the impaired performance is truly a result of greater LTP saturation in those animals who are closer to their synaptic ceilings to begin with, then the correlation should disappear if training takes place first and all animals should perform equally well in the watermaze. On the other hand, if both the impaired performance and the lower LTP levels result from some characteristic property of the synapses, intrinsic to the animals, then the correlation should be preserved.

The observation in Experiments 1 and 2 of a correlation between LTP accumulation and spatial learning ability recalls the findings of Barnes (1979) and Barnes and McNaughton (1985) that LTP correlates with learning after a few days of tetanisation. It differs from their findings, however, in that in Experiment 2 the final levels of LTP still differed between good and poor learners even after a prolonged period of daily tetanisation, whereas LTP in the poor learners of the Barnes and McNaughton study had caught up with that of the good learners by the 12th day of tetanisation. It is unlikely that the lower LTP levels seen in the poor spatial learners in the present experiments are an artefact of slower LTP acquisition such as that observed by Barnes and McNaughton, because comparison of the acquisition of EPSP LTP for the 3 good and 5 poor learners (Fig. 3.9) suggests that poor learners appear to have reached asymptote *sooner* than the good learners, and at a lower overall level of LTP. Thus, the apparent difference in final LTP does not seem to be an artefact of varying acquisition time-constants. This question could be resolved by a comparison of overnight LTP decay between good and poor learners.

It is not clear why Barnes and McNaughton failed to see an LTP/learning correlation such as those observed in Experiments 1 and 2. One possibility is that the LTP protocol that they used succeeded in forcing synaptic strengths up to their ceiling, whereas those used here failed to achieve true saturation. Thus, the variability in cumulative LTP levels seen in the present experiments may have been masked, in their study, by a saturation effect. In this context it is worth noting that the same authors have produced the only studies reporting a spatial learning impairment following LTP induction (McNaughton *et al.*, 1986, Castro *et al.*, 1989). If this is the case, then the association of LTP with learning seen in Experiments 1 and 2 should begin to disappear as synaptic strengths approach their upper limits. An alternative possibility is that learning may depend on an interaction between both the magnitude and the durability of LTP, and that only the latter varied between the young and old rats in the Barnes and McNaughton (1985) study. The behavioural protocol in their experiments involved training over several days whereas the experiments reported here required the rats to remember information only for minutes to hours. Possibly LTP induction contributes to acquisition of a spatial task (learning) whereas its maintenance contributes to retention (memory). It may be possible to

dissociate the relative contributions of these two parameters by means of pharmacological manipulation of LTP induction (*e.g.* with NMDA-receptor blockade) and maintenance (*e.g.* with sodium pentobarbital, Jeffery *et al.,* 1990).

A final point raised by the findings of Experiments 1 and 2 is that although an apparent asymptote was reached by both tetanisation protocols, the level of maximum LTP attained in each case was different (30% *vs.* 50%). The possibility arises that asymptote is not an appropriate measure of true saturation, and that the failure of the previous experiments to see an effect of such so-called "saturation" on learning may have been due to a residual capacity for potentiation being able to mediate spatial learning.

The present experiment addressed three of the issues raised by the finding of the previous experiments as follows:

(1) Did LTP induction produce the learning distribution? Conversely, does the learning/LTP relationship persist if the order of training and tetanisation is reversed?

(2) Is there a difference in decay rate between good and poor learners? If so, would this explain the apparent accumulation of LTP in good as opposed to poor learners?

(3) Does the asymptote seen after repeated stimulation represent a true maximum?

Did LTP induction produce the learning distribution?

First, if both the maximum level of LTP induction (the "LTP ceiling") and the spatial learning ability of a given rat arise from the plastic properties of its synapses, then the LTP-learning correlation should be reproducible if training were to precede tetanisation. On the other hand, the distribution of spatial learning ability seen in Experiments 1 and 2 may have come about because those rats in which higher LTP levels had been induced had been conferred a proportional physiological advantage. In other words, rats achieving the most LTP as a result of the tetanisation protocol may have become betters learners than those getting less LTP, because LTP induction somehow improves learning. As mentioned in Chapter 1, such a tetanisation-induced facilitation of learning has been reported for a non-spatial task in rabbits (Berger, 1984). Conversely, the distribution of performance may have arisen because the surgical and electrophysiological intervention had damaged either the perforant path fibres or the dentate gyrus itself, which could have resulted in a parallel impairment of both LTP and spatial learning even in the absence of a direct causal link between the two phenomena. If the experimental protocol was affecting learning, however, then either of these two possibilities would be expected to manifest themselves as a change in group mean learning performance between the experimental group and the controls. The finding that overall spatial learning performance did not differ in rats which were tetanised, given low frequency stimulation or not operated upon at all suggests that either of these explanations would be unlikely, though not impossible.

However it is a simple hypothesis to test: if either proportional facilitation or proportional impairment of learning had been responsible for the LTP-behaviour correlation, then if training were to precede tetanisation the correlation should disappear and all rats should perform equally well in the watermaze, regardless of their *subsequent* LTP levels. Thus, in the current experiment, the methodology of Experiment 1 was replicated but in half the animals the order of training and tetanisation was reversed.

Does the decay rate differ between good and poor learners?

Second, the correlation may have arisen not because the maximum sustainable level of LTP was lower in poor learners, but because LTP acquisition in these animals was so slow that after 5 or even 14 consecutive days of tetanisation they still had not reached ceiling LTP levels. One possible reason for slow acquisition was suggested by the findings of Barnes (1979) and Barnes and McNaughton (1985): namely, that animals in which overnight LTP decay was more rapid quickly fell behind in their accumulated total. The animals possessing faster LTP decay were older animals in which spatial learning also showed a proportional impairment. In those studies, continuation of daily tetanisation out to 12 consecutive days overcame the slower acquisition and final, asymptotic levels were equal between old and young, and good and poor learners. Thus, it is possible that if tetanisation had been continued in the experiments reported here, LTP in the poor learners would finally have caught up with that in the good learners.

This possibility was tested in two ways in Experiment 3. First, if faster LTP decay was the reason for the low level of LTP gained by poor learners in Experiments 1 and 2, then continuation of the period of tetanisation for more days should show that LTP in these animals was indeed still increasing. Therefore, in this experiment the LTP protocol used in Experiment 1 was continued for a further 3 days, to allow comparison of the final value with that seen after only 5 days of tetanisation. However, the pattern of LTP acquisition depicted in Fig. 3.9 suggests that if LTP in the poor learners is truly heading towards the same asymptote as that of the good learners, the acquisition time constant would have to be considerably lower in the animals used here than in the Barnes and McNaughton study. Extrapolation from the data points in Fig. 3.9 suggests that the two curves would only meet after many days of tetanisation, rather than the 12 days observed by Barnes and McNaughton. This may be a reason for doubting that faster decay/slower acquisition could be the explanation for the lower LTP levels in the poor learning rats in this study. However, it may be that for some reason the LTP induction protocol used here results in slower LTP acquisition. The other, more direct approach is to measure the decay rate itself. This was attempted after tetanisation for the 8 animals in Experiment 2, but the results were difficult to interpret because of the small numbers of animals and the variability of day-to-day evoked potentials. Consequently, in Experiment 3, only

overnight decay was measured. If faster overnight decay was slowing the LTP acquisition rate in poor learners this should be detectable by a simple measurement of the pre-tetanisation evoked potential each day.

Does the asymptote represent a true maximum?

The third issue is raised by the observation that the final level of LTP gained in Experiment 2, after 14 days of tetanisation, was higher than that gained by the animals in Experiment 1 after 5 days of tetanisation. This is of interest because the animals receiving only 5 days of tetanisation nevertheless received a higher total number of trains (250 as opposed to 140). The possibility arises that intense tetanic stimulation might somehow constrain the amount of LTP able to be induced. The alternative possibility is simply that the apparent LTP plateau seen in Experiment 1 is spurious, and that had daily tetanisation been continued for several more days the level would have continued to rise.

4.2 Experiment 3 Methods

The time course of this experiment is shown in Fig. 4.1. Sixteen rats were divided into two groups, WM-LTP (n = 8) and LTP-WM (n = 8), to receive training before or after tetanisation, respectively. A third group of unoperated controls (UC, n = 8) was included to provide a measure of baseline performance.

4.2.1 Surgery

Sixteen rats were implanted with perforant path and dentate gyrus electrodes bilaterally, as described in the General Methods. The electrodes were bipolar with tips separated vertically by 0.5 mm.

WM-LTP	surgery + recovery	pretraining	baseline 5 days	training	tetanisation 8 days 50 trains	rest day	tetanisation 9 days 10 trains
LTP-WM	surgery + recovery		baseline 5 days	pretraining	tetanisation 8 days 50 trains	training	tetanisation 9 days 10 trains
UC		pretraining	handling 5 days	training	handling 8 days		
			handling 5 days	pretraining	handling 8 days	training	

Figure 4.1 Design of Experiment 3.

4.2.2 Electrophysiology and behavioural training

After 2-6 weeks' recovery, evoked potentials were checked, stimulus intensities determined (so as to evoke a 1-3 mV population spike) and the implanted rats assigned randomly to either the WM-LTP or LTP-WM groups. On the day before the first baseline electrophysiology session, rats from the WM-LTP group and half of the unoperated controls were given 6 pretraining trials in the watermaze while those from the LTP-WM group remained in their home cages. Baseline recording began the next day and was continued for 5 days. Each unoperated rat was paired with an experimental rat and received equivalent handling during this time. The following day the WM-LTP group and the pretrained unoperated controls underwent spatial training in the watermaze according to protocol 1, while the LTP-WM rats and the remaining controls stayed in their home cages except for 6 pretraining trials. The next day the tetanisation phase of the experiment began. All implanted rats were given tetanic stimulation according to HF protocol 1, for 8 days. The following day the rats which had not yet received spatial training (the LTP-WM group and remaining controls) were trained in the watermaze according to the same training regimen (protocol 1) while the WM-LTP rats remained in their home cages. Thus all implanted rats had received the same amount of tetanisation but half were trained before and half after the LTP induction phase.

Following this phase of the experiment, the tetanisation intensity was reduced to 10 trains per day, using a spaced protocol (HF protocol 3) and continued for a further 9 days. Eight days after the last tetanisation session a final measurement of evoked potentials was made, to assess long-term decay.

4.3 Experiment 3 Results

The evoked potentials from 1 rat in the WM-LTP group deteriorated during tetanisation and all data from this rat have been excluded. There are therefore 7 WM-LTP rats and 8 LTP-WM rats.

4.3.1 Behaviour

All 3 groups showed a significant improvement in latency across the training trials [$F(7,14) = 26.83$, $p < 0.0001$] and there was no difference between groups [$F(14,140) < 1$, NS; Fig. 4.2A]. The rats spent significantly more time in the training quadrant during the absent-platform test [$F(3,6) = 37.41$, $p < 0.0001$] with no difference between groups [$F(6,60) < 1$, NS; Fig. 4.2 B].

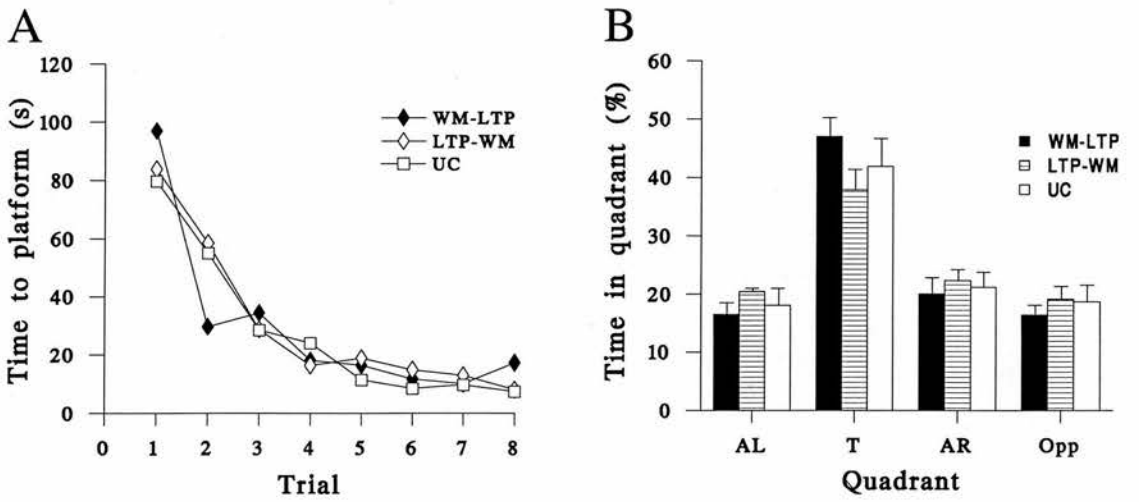


Figure 4.2 (A) Latency data for the 8 watermaze trials for the WM-LTP, LTP-WM and UC groups. Training was conducted on either the day after baseline electrophysiological recording (WM-LTP group) or the day after the 8-day period of 50-train tetanisation (LTP-WM group). (B) Absent-platform test 2 min after the last trial (group mean % quadrant time ± SEM).

4.3.2 Electrophysiology

After the spatial learning performance had been ascertained, the 15 rats from both WM-LTP and LTP-WM groups were divided into good and poor learners depending on whether they were above or below the group mean, respectively, on the absent-platform test. Thus, electrophysiological results described below have been grouped in three ways: (1) all rats, (2) according to tetanisation condition (before or after training for LTP-WM and WM-LTP groups, respectively) and (3) learning ability (good and poor learners, being above and below the group mean respectively).

Raw values for stimulation intensity, baseline and post-tetanisation EPSP and spike and training quadrant times are shown in Table 4.1.

All rats

LTP acquisition for the EPSP and population spike is shown for all 15 rats in Figs. 4.3A,B. The 8 days of tetanisation at 50 burst trains daily produced apparently asymptotic LTP for both parameters (days 7-14). When the stimulation was reduced to 10 spaced trains there was a further increase for the EPSP but not the population spike.

The experimental period was then divided into 4 blocks according to the type of stimulation administered: baseline, 50 trains/day, 10 trains/day or decay. These data are shown in Fig. 4.3. A one-tailed paired *t*-test between the mean of the last 6 days of the 50-train block and of the last 6 days of the 10-train block revealed a highly significant increase in the size of the EPSP ($t = -3.34$, $p < 0.01$) but not the population spike ($t = 0.28$, NS).

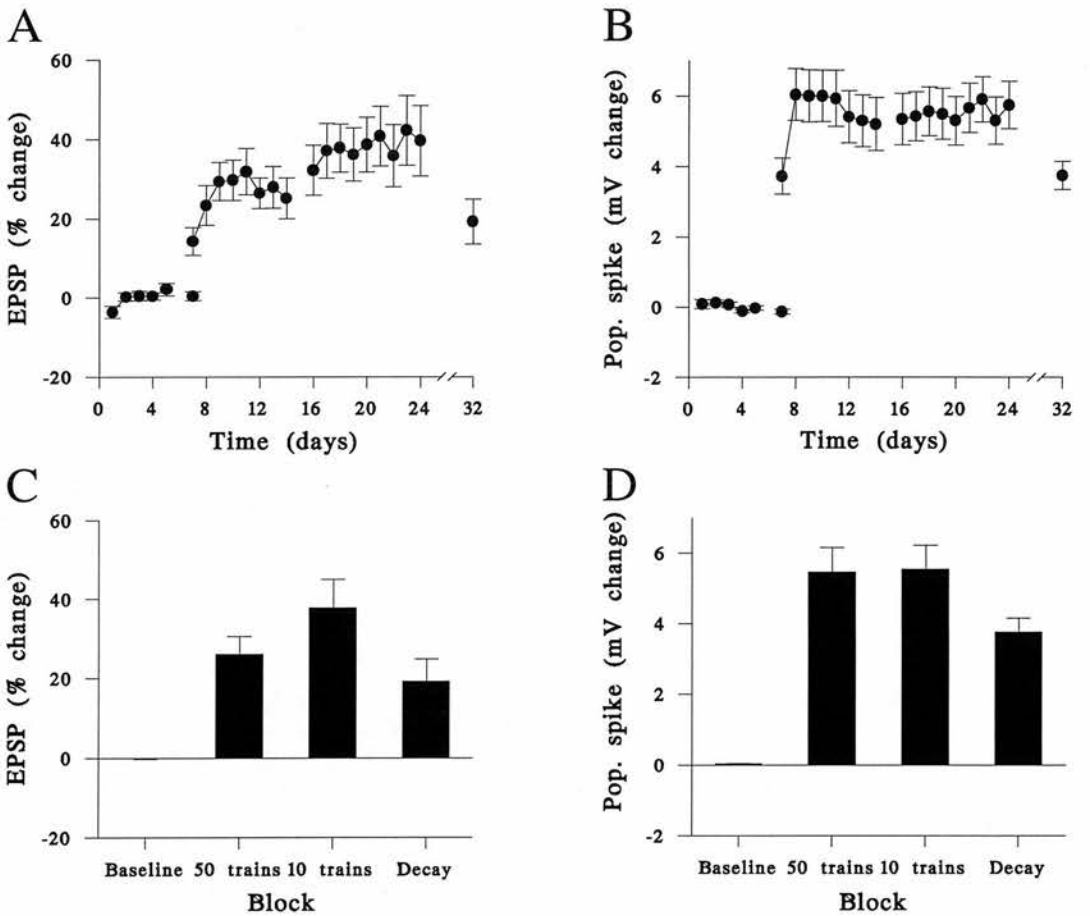


Figure 4.3 All rats: normalised values for the EPSP (A) and population spike (B) following several days of high-frequency stimulation. Days 1-5, baseline. Day 6, water maze training or rest day. Days 7-14, high-frequency stimulation 50 trains daily. Day 15, rest day or water maze training. Days 16-24, high-frequency stimulation (10 trains daily). Day 32, final baseline 8 days after last high-frequency session. (C) and (D): the data shown in A and B grouped into blocks according to the type of stimulation administered. Values for the baseline period are close to zero because of the normalisation process, but are included to allow easier visual comparison of these phases with the phases shown in the graphs above. The EPSP (C) showed a significant increase when the stimulation was reduced from 50 burst trains to 10 spaced trains daily, whereas the spike (D) showed no change.

	Stimulus intensity (μA)	Baseline EPSP (mV/ms)	Baseline spike (mV)	Final EPSP (mV/ms)	Final spike (mV)	Training quadrant time (%)
All rats (n = 15)	328 (32)	4.27 (0.28)	2.11 (0.19)	5.24 (0.33)	7.29 (0.64)	42.1 (2.6)
WM-LTP (n = 7)	329 (50)	3.92 (0.49)	2.37 (0.28)	4.68 (0.60)	6.84 (0.95)	46.9 (3.3)
LTP-WM (n = 8)	327 (43)	4.57 (0.28)	1.88 (0.25)	5.73 (0.25)	7.69 (0.91)	37.9 (3.43)
poor learners (n = 7)	381 (50)	4.56 (0.35)	2.32 (0.36)	5.39 (0.50)	6.59 (0.72)	33.3 (2.0)
good learners (n = 8)	281 (36)	4.00 (0.42)	1.93 (0.17)	5.10 (0.46)	7.90 (1.02)	49.9 (2.0)

Table 4.1 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after 8 days of tetanisation. Rats are also divided according to both group and spatial learning ability. Also shown is the measure of spatial learning ability (time spent in training quadrant).

WM-LTP and LTP-WM groups

Acquisition of LTP for the rats trained before tetanisation (WM-LTP) as compared with those trained after (LTP-WM) is shown in Fig. 4.4.

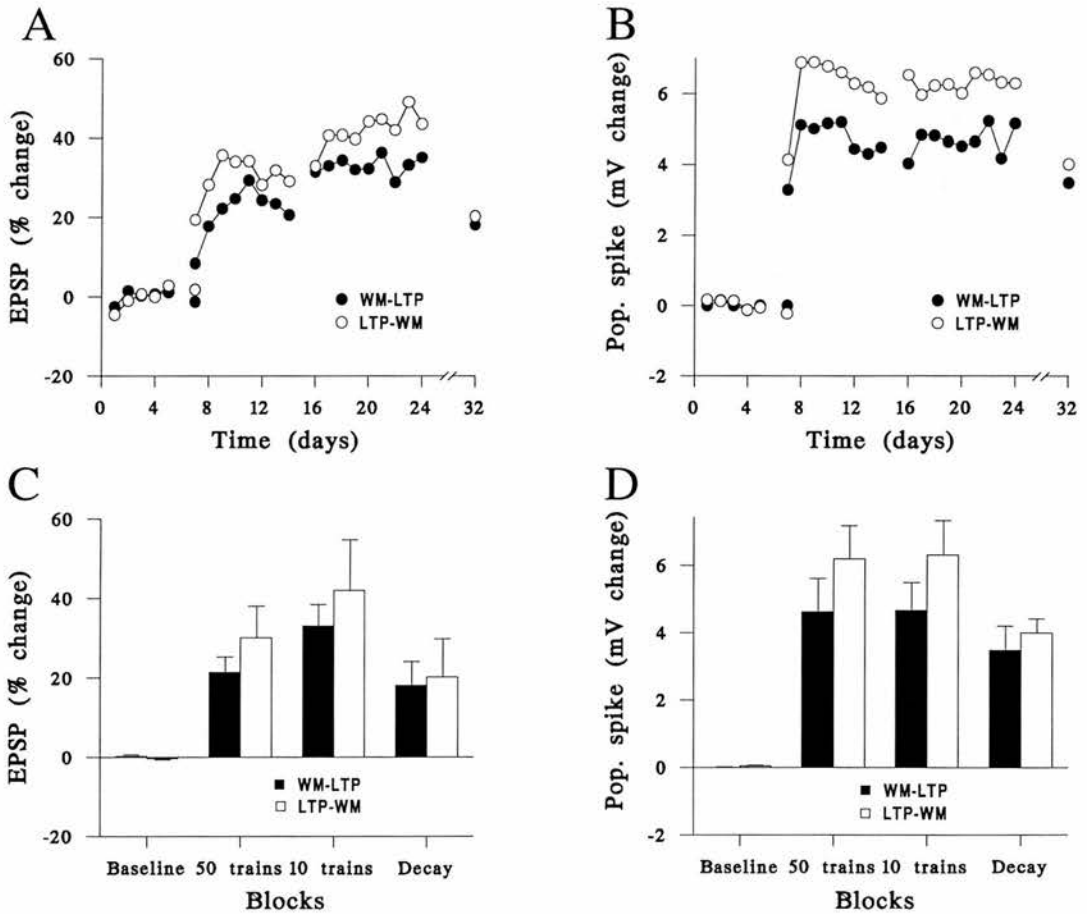


Figure 4.4 LTP acquisition of the EPSP (A, C) and the population spike (B, D) when the rats are grouped according to whether they were trained before or after tetanisation.

The pre-tetanisation values on the first tetanisation day did not differ between rats which had been trained the previous day and those which had rested in their home cages [$F(1,13) = 1.92$, NS], suggesting that if there were any training-induced changes in perforant path physiology they did not manifest themselves as changes in the evoked response. Rats trained first showed less LTP of both the EPSP and the population spike, but this was not significant for either the 5th, 8th or final (17th) day of tetanisation [F's all < 1, NS].

Good and poor learners

The 15 rats were divided on the basis of their performance on the absent-platform test into poor (n = 7) and good (n = 8) learners (above and below the group mean, respectively) and evoked potentials compared between the two groups. LTP acquisition for the rats divided into good and poor learners is shown in Fig. 4.5.

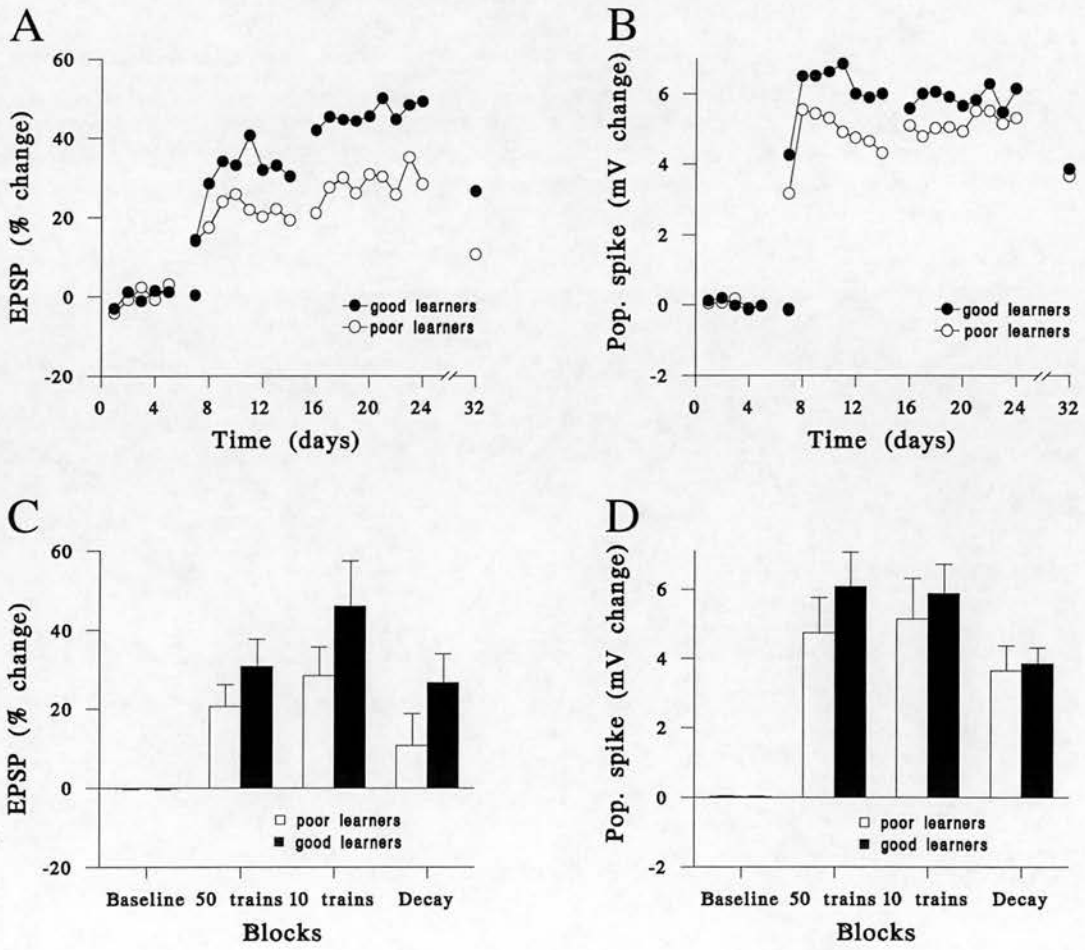


Figure 4.5 LTP acquisition of the EPSP (A, C) and the population spike (B, D) when the rats are divided into poor and good learners. Good learners achieved more LTP but this was not significant.

Comparison of the individual scores on the absent-platform test with LTP after 5 days of tetanisation is shown in Fig. 4.6. The analysis was performed using the day 5 data point because these were the values used in Experiment 1, and also because this was the day at which the LTP acquisition curves differed most between good and poor learners (Fig. 4.5A,B). There was no significant correlation between these values for either the EPSP ($r = 0.27$, NS) or population spike ($r = 0.36$, NS). The analysis was repeated using the day 8 or day 17 tetanisation days and still did not reach significance (r's all < 0.35, NS).

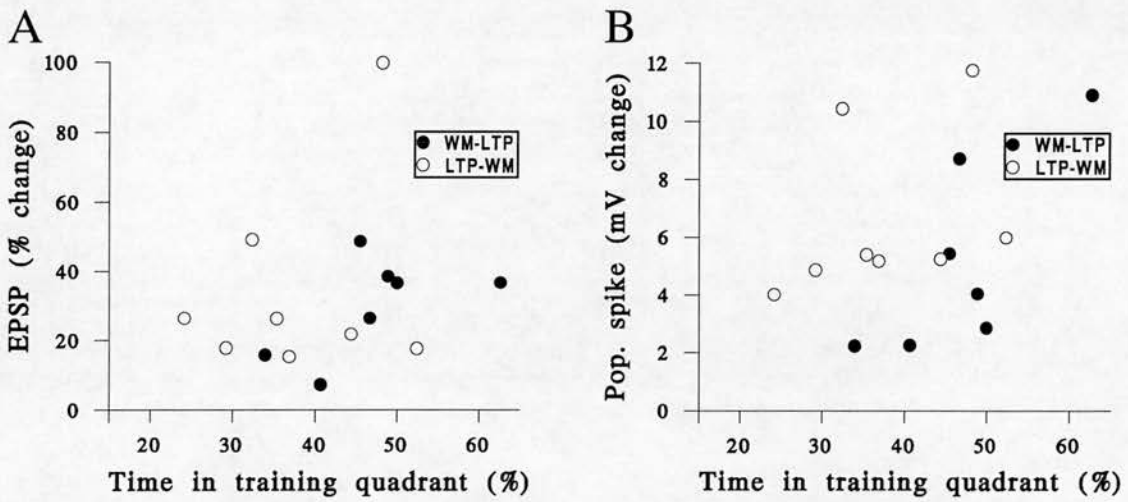


Figure 4.6 Comparison of spatial learning performance with potentiation of the EPSP(A) and population spike (B) after 5 days of tetanisation. There was a trend towards a positive correlation only in the rats trained first (WM-LTP group) for both EPSP and population spike but this was not statistically significant. The potentiation after 8 or 17 days of daily tetanisation showed a similar pattern.

Daily LTP acquisition and decay for the good and poor learners is shown in Fig. 4.7. This was quantified by summing the daily increments and the overnight decrements for each rat. Analysis of variance of the total LTP increment revealed no significant difference between the good and poor learners for either EPSP [$F(1,13) < 1$, NS] or population spike [$F(1,13) = 2.34$, NS]. Likewise, there was no difference in total LTP decay between the two groups for the EPSP [$F(1,13 < 1$, NS] or population spike [$F(1,13) = 2.55$, NS]. In fact the totals for poor learning rats were, if anything, slightly higher than good learning rats for acquisition and even more so for decay (Fig. 4.8), suggesting that the deficit in LTP seen in the poor learning rats probably did not result from slower LTP acquisition.

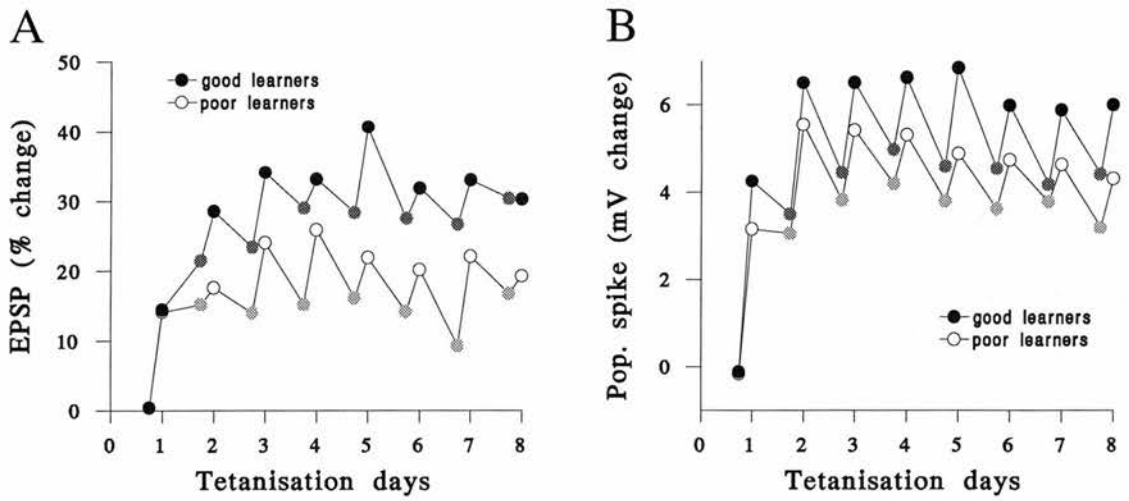


Figure 4.7 Expanded view of the 50-train tetanisation phase, showing overnight decay (grey circles) prior to each tetanisation session.

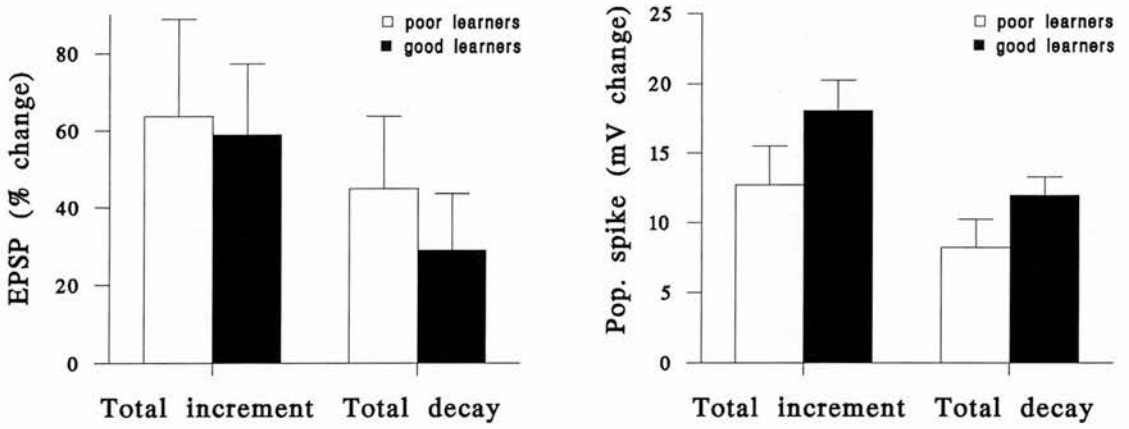


Figure 4.8 Total LTP acquisition and decay for good and poor learners. The values were obtained by summing the daily % LTP acquisition and decay for the 50-train tetanisation phase.

4.4 Discussion of Experiment 3

There were three aims of Experiment 3, which will be discussed in turn. First, the experiment was intended to investigate whether the LTP/learning correlation seen in the first two experiments persisted if training preceded tetanisation instead of following it. Somewhat unexpectedly, the correlation was not seen in the animals where training *followed* tetanisation, so no firm conclusion can be drawn about whether LTP may or may not have been affecting learning in the previous experiments.

Second, the amount of daily LTP decay was recorded in order to determine whether an accelerated decay process such as that reported by Barnes (1979) and Barnes and McNaughton (1985) could explain the slower LTP acquisition seen in the poor learning rats in Experiments 1 and 2. Because LTP was not significantly lower in the poor learning

rats in this experiment, again it is not possible to draw any firm conclusions. However a tentative conclusion suggests itself and this will be discussed below.

Finally, after LTP asymptote had been reached using a strong induction protocol, tetanisation intensity was reduced and tetanisation sessions continued for several more days. The purpose of this was determine whether the original asymptote represented a true ceiling for LTP, or whether it could be surmounted under some circumstances.

4.4.1 Correlation if training precedes tetanisation?

The reasons why a post-tetanisation correlation of LTP with learning may not have been seen in the present experiment are as follows. First, the correlations in Experiments 1 and 2 may have been spurious. That is, because only a small number of animals were used ($n = 8$) in each experiment, the correlations may have been due to chance. The low *p*-values associated with those results ($p < 0.001$ and $p < 0.05$ for the EPSPs in Experiments 1 and 2 respectively) make this somewhat doubtful. The power of this observation is reduced by the fact that the correlation in the Experiment 1 was not explicitly sought. However, the correlation in Experiment 2 *was* explicitly sought and still reached statistical significance. Another reason for believing that the correlation is not simply due to chance is that a hint of an LTP/learning association is seen for the animals trained first in the correlograms in Fig. 4.6 of this chapter. However, it is not clear why the correlation should be more apparent for the animals trained first (the WM-LTP group) when the previous correlations had been seen in animals trained after tetanisation. The procedure used in Experiments 1 and 2 was followed as closely as possible in this experiment.

One possibility is that a selection bias was operating in the other direction: that is, an underlying difference between good and poor learners was inadvertently obscured either by subject selection or by some feature of the experimental protocol, leading to a Type II statistical error. There is some suggestion that the good and poor learning groups differed in a way that was not necessarily related to underlying synaptic plasticity: for example, the poor animals were tetanised and tested with, on average, a slightly higher stimulus intensity than the good learners (Table 4.1). It may be that the rats in the poor learning group happened to differ in some baseline parameter, such as dentate gyrus excitability, leading to a difference in LTP acquisition. Alternatively, the selection of a slightly higher test stimulus intensity for these rats might have inadvertently positioned the LTP measurement point on a different part of the IO curve. If there is a difference in IO curve shape after tetanisation between poor and good learning rats then this might result in artefactually different LTP measurements, thus obscuring a real variation in synaptic plasticity between the two groups. This very important point will be addressed in the next chapter. As will be seen, a variant of this explanation is in fact the most likely reason for

the failure of the present experiment to see a clear separation in LTP acquisition between good and poor learners.

4.4.2 Do decay rates differ between good and poor learners?

As well as invalidating arguments concerning the effect of LTP induction on spatial learning performance, the absence of the LTP/learning correlation in this experiment also reduces the power of any inferences on the effects of decay rate on the accumulation of potentiation. However some trends are apparent from the data seen here. First, although the differences did not reach statistical significance, the poor learners did achieve less LTP than good learners (Fig. 4.5). Assuming for the moment that this difference reflected an underlying LTP/learning correlation which was for some reason partially obscured, it is worth examining how much of this difference resulted from less LTP acquisition and how much from faster overnight decay. The decay time course is shown in more detail in Fig. 4.7. The amount of LTP resulting from the first tetanisation session was essentially identical in the two groups. LTP acquisition and decay following repeated tetanisation were described by comparing the total amount of accumulation and decay. If poor learners were showing less accumulated LTP because of slower acquisition or a lower ceiling then the values for both LTP acquisition and decay should be small, because decay is an exponential process and the amount of decay would be proportional to the amount of potentiation (assuming equal decay time constants for the two groups). On the other hand, if poor learners were acquiring LTP at the same rate and their lesser accumulation was due to faster decay, then the values for summed acquisition and summed decay should be as large as (if not even larger than) the values for the good learners. The actual values shown in Fig. 4.7 indicate that the poor learners gained, overall, slightly more LTP than the good learners, as well as showing slightly more decay. Neither of these values reached significance, so no more can be concluded than that there is a trend towards faster decay of LTP in the poor learning rats which may explain why they showed less LTP, and may, by inference, underlie the correlation seen in the first two experiments.

4.4.3 Does the LTP asymptote represent a true maximum?

The final question addressed by this experiment concerned whether the asymptote reached after repeated tetanisation is truly a maximum, or whether it represents a local ceiling for LTP which could be surmounted under the appropriate circumstances. This question was prompted by the observation in the previous two experiments that the LTP maximum was considerably higher when fewer trains of stimulation were given over a longer period of time. The results of Experiment 3 suggest that more intense stimulation may result in a lower ceiling for LTP than less intense stimulation, for the EPSP but not the population spike (Fig. 4.3). After 8 days of tetanisation at 50 burst trains daily, LTP of

the EPSP appeared to have reached a clear plateau and was, if anything, declining. When the protocol was changed to 10 spaced trains daily the EPSP began to increase again and stabilised at a significantly higher level. This finding has implications for saturation studies of the kind addressed in Part I. If an apparent ceiling can be overcome by changing the stimulation conditions then the question arises as to whether *any* ceiling can ever be considered, with confidence, to be the true ceiling for that population of synapses, or whether there might always be some other stimulation protocol which could drive synaptic strengths higher still. It therefore appears that asymptote is an invalid measure of saturation.

The reason for the impediment to further LTP acquisition produced by more intense stimulation is currently unknown, but because the stimulation received by rats in the 10-train phase was a *subset* of the stimulation they received during the 50-train phase (*i.e.* for every 5 trains they received in the latter they received only 1 in the former) only two classes of possibility exist. Either the last 40 trains of the 50 train protocol counteracted the effect of the first 10 by erasing some of the message the postsynaptic cells need to establish LTP, or they triggered an additional process the end result of which was to diminish the size of the EPSP (though not the population spike) and produce an apparent, rather than real, decrease in LTP. These two possibilities will be considered in turn.

The first possibility may involve the consolidation processes acting to convert STP into LTP. After a single tetanus, a sequence of cellular events is activated which involves postsynaptic second messenger systems and culminates in the expression of LTP. It appears that if subsequent trains follow after a sufficiently long interval (minutes to hours) then LTP will accumulate, whereas if they follow too closely (seconds) then further increases will be limited. Subsequent trains (each arriving, in the present case, one second after the previous one) trigger the same cycle of postsynaptic depolarisation, NMDA receptor activation, Ca^{2+} influx, second messenger signalling and so on. It may be that if the second tetanus arrives while the first is still being processed (say, for example, by translocation of protein kinase C) then the two will not be additive. If the second arrives after the first has been fully processed and LTP established, then the two *will* be additive. This finding has certain important consequences for the information-processing function of LTP, because it places a limit on the type of temporal integration which may occur. An analogy suggests itself with psychological processes such as retroactive interference and the superiority of distributed over massed trials in promoting learning. However, whether the capping of LTP associated with closely spaced stimulation is the physiological basis for these phenomena cannot be decided at present.

The second possibility is that under the 50 burst trains condition, repeated stimulation at short intervals activates a second process which counteracts the increase in the EPSP

produced by LTP. There is some suggestion that such a counteractive process may be in operation in the observation that during the latter part of the 50-train condition the EPSP slope decreased slightly each day (Fig. 4.3). This decrease is unlikely to have been due to damage to perforant path fibres following from the intense stimulation, because it was immediately erased on commencement of the 10-train protocol and the EPSP began to grow again. Two candidate counteractive processes are feedforward inhibition from the local basket cell network and long-term depression (LTD).

Excitatory synaptic contacts onto feedforward inhibitory cells are thought to be potentiable in the same way as are the contacts onto granule cells (Kairiss *et al.,* 1987). If the former type of excitatory/inhibitory potentiation had a higher threshold than the latter, it may be that 50 trains would also potentiate inhibition whereas 10 trains would only potentiate excitatory/excitatory synapses. However, potentiation of inhibition would be unlikely to affect the rising phase of the field potential, which has a latency too short to be influenced by IPSPs (though it may affect the population spike, see below). LTD, on the other hand, is likely to occur alongside LTP and therefore to counteract potentiated EPSPs directly. Two types of LTD are though to occur in the hippocampus: heterosynaptic LTD, which follows from simple tetanisation (Lynch *et al.,* 1977, Abraham and Goddard, 1983) and homosynaptic LTD, whose existence is still disputed but which may result from the arrival of a train of pulses at a time when the postsynaptic cells are hyperpolarised (Stanton and Sejnowski, 1989). Heterosynaptic LTD occurs in non-stimulated fibres but may also involve those which transmitted the tetanus, resulting in less apparent LTP than would have occurred had the concomitant depression not been triggered (Abraham *et al.,* 1985). Homosynaptic LTD involves only the fibres which were stimulated. It has proved somewhat difficult to establish with confidence, but it may be an occult accompaniment of LTP and act to reduce the size of the EPSP increase. It may be that the repeated application of trains at 1 s intervals means that only the first train arrives at resting postsynaptic cells, whereas the remainder arrive during the period of post-tetanic afterhyperpolarisation and hence trigger LTD formation.

Reducing the number of trains effectively produced a right-shift of the E-S relationship, because the EPSP continued to grow daily but the population spike stayed at the same level. It can be concluded from this finding that no extra granule cells were being brought to firing threshold by the increased postsynaptic depolarisation. There are two possible explanations for this. One is that repeated tetanisation using the 10-train condition may be reducing granule cell excitability by exactly the same amount as the EPSP is increasing each day, so that the total number of granule cells firing stays constant. This could happen if, for example, excitatory synapses onto feedforward inhibitory cells were being potentiated at the same rate as excitatory synapses onto the granule cells themselves. Another reason is that the extra potentiation procured by the new stimulus protocol

involved synapses which contact cells which were already being brought to threshold by the perforant path stimuli. Thus, although they contribute to greater cell depolarisation, they may not produce more action potentials than would already have been generated by the existing depolarisation.

4.5 Conclusion of Experiment 3

Experiment 3 produced a positive finding, that asymptotic LTP can be surmounted if the stimulation is reduced, suggesting that asymptote may not be a valid measure of "saturation". Two other questions addressed by the experiment remain unanswered: that is, whether the correlation between LTP and learning seen in Experiments 1 and 2 can be replicated if the order of training and tetanisation is reversed, and whether the correlation resulted from variations in the durability, acquisition rate or maximum sustainable level of LTP. The failure to see the correlation in this experiment is puzzling, given its strength and reproducibility in Experiments 1 and 2 and the fact that the present conditions were matched as closely as possible with those of the first experiment. One possible reason is that the correlation is sensitive to the position on the IO curve where LTP is measured. This possibility is addressed by the experiment described in the next chapter.

Chapter 5 – Experiments 4 and 5

5.1 Introduction to Experiment 4

The aim of Experiment 4 was to determine whether the absence of a significant LTP/learning correlation in Experiment 3 might have been due to some feature of the experimental protocol which either (a) produced an apparent but spurious correlation in the first two experiments, or (b) inadvertently masked an underlying real LTP/learning correlation in Experiment 3. The ways in which either of these two possibilities could account for the results of the preceding three experiments are as follows.

5.1.1 Spurious initial correlation

The first possible explanation for the findings to date is that the correlation found in the first two experiments was not due to differences in synaptic plasticity between the two groups, but came about as an artefact of some other difference between good and poor learners. For example, there may have been differences in baseline responsiveness between good and poor learners which produced a difference in subsequent measured LTP. There are two ways this could have happened: either these excitability differences resulted in a true difference in the amount of LTP being induced in good versus poor learners, or the overall amount of LTP induced was the same in both groups but was measured to be different because of different baseline test stimulus intensities.

Different LTP induction between good and poor learners

An undetected difference in the baseline responsiveness between good and poor learners might have resulted in a secondary difference in LTP as follows. Recall that both tetanisation and test stimulus intensity were set according to a criterion (1-3 mV) determined by the population spike independently of the EPSP. If there was an excitability difference such that a population spike was more readily elicited in one group of animals, then the two groups might have required different stimulus intensities to evoke a spike of the requisite size, thus modulating the amount of subsequent LTP. It is not clear in which direction this relationship should lie: an animal with an excitable dentate gyrus might have required a smaller stimulus intensity, thus leaving open the possibility that some perforant fibres would not be stimulated sufficiently strongly to generate action potentials and the presynaptic activity needed to trigger LTP induction, with resulting lower population LTP levels. However, it was shown in the preceding chapter that a less intense stimulation paradigm might paradoxically result in a greater

accumulation of LTP. Thus, an argument could be constructed to defend either prediction. Nevertheless, it appears plausible that a difference tetanisation stimulus intensity could result in a difference in LTP induction between good and poor learners. Arguing against this, there appeared to be no such difference in the two experiments in which the correlation was observed (Tables 3.2 and 3.4). An alternative and closely related possibility is that although stimulus intensities were matched between the two groups, a baseline excitability difference resulted in a larger population spike in one group, with consequent facilitated LTP induction. Again, however, there appeared to be no difference in this parameter between good and poor learners. Nevertheless, until the responses are compared across a range of stimulus intensities it cannot be asserted with confidence that good and poor learners did not differ before tetanisation.

Difference in measured LTP between good and poor learners

Second, it could be that the changes in the input-output relationship after LTP induction might produce spurious differences in the amount of LTP *measured* (though not necessarily actually induced) between good and poor learners. For example, the percentage of LTP induction occurring might vary across the IO curve, so that a measurement at one point on the curve would give a different value of LTP from that measured elsewhere (Fig. 5.1). It has been noted that LTP does not necessarily occur in equal proportion across the IO curve but is lower with high test intensities (Cain *et al.*, 1993).

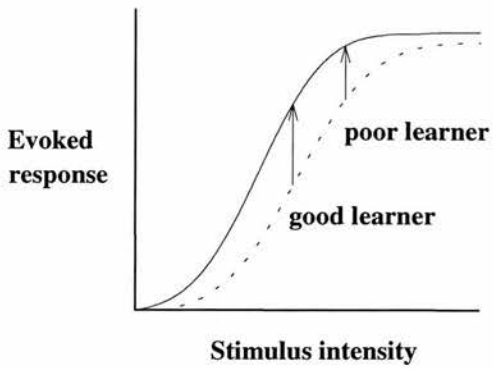


Figure 5.1 Schematic IO curves showing how variations in measurement position could produce an artefactual variation in the amount of measured LTP. Dotted line = baseline, solid line = post-tetanisation. If LTP was not proportionally constant across the curve but declined with increasing test currents, then a bias towards stronger test stimulus intensities in poor learning rats would result in LTP in these animals being underestimated.

If, for some reason, poor learners were consistently being measured at a different point on the curve (they might, for example, have required a higher stimulus intensity to evoke the 1 mV population spike used to set the baseline stimulus intensity, as discussed above)

then this could result in an apparent decrease in the measured amount of LTP, even if the whole IO curve actually shifted the same amount between good and poor learners

5.1.2 Valid initial correlation and obscured subsequent correlation

An alternative explanation for the contradictory results of the preceding three experiments is that a dependence of measured LTP on baseline stimulus intensity might have acted to obscure, in Experiment 3, a real difference which had been detected in Experiments 1 and 2. Possible ways in which this may have come about involve the type of LTP-modulating factors discussed above, but acting to mask a real correlation rather than induce a spurious one. For example, a slight and accidental inequality between good and poor learners in the baseline stimulus intensities used to induce and measure LTP, or in baseline evoked responses, might have produced a depolarisation-dependent disruption of LTP. Supporting this argument, the poor learners in Experiment 3 were tetanised with a higher stimulus intensity and had larger pre-tetanisation evoked responses. Either of these factors may have caused these animals to gain larger LTP than they otherwise would have, thus obscuring an underlying difference in "LTP-ability".

If it is assumed that LTP induction *does* correlate with learning given the right circumstances, it may be the case that both LTP and spatial learning ability do not share a common basis but rather are independently modulated by ascending non-specific influences such as arousal, alertness or stress. If this were so then the correlation of LTP with behaviour would be likely to extend beyond the spatial domain to include other forms of learning. In addition, even if the LTP-learning link were truly related to underlying synaptic plasticity, the observation of a relationship between specifically hippocampal LTP and a specifically hippocampal task (*i.e.* spatial learning) could have been coincidental and the level of LTP measured anywhere in the brain might have correlated with performance on any selected task. In other words, there may be a link between general cognitive ability and plasticity of synapses throughout the brain. The corollary to this is that the finding of a specific correlation between hippocampal LTP and performance on a spatial task would constitute further evidence that the spatial learning function of the hippocampus may be subsumed by the plasticity of its synapses. To demonstrate such specificity, it would be necessary to find dissociations between hippocampal LTP and performance on other, non-spatial tasks, or at the very least to determine whether spatial learning ability is inextricably linked to learning in other domains or can be separated from them.

5.1.3 Aims of Experiment 4

Experiment 4 had two main aims. First, it set out to test the hypothesis that the correlation discovered in Experiments 1 and 2 could be replicated if it could be ascertained with

confidence that input-output relationships were constant between good and poor learners prior to tetanisation. The conditions of Experiments 1 and 3 were therefore replicated except that LTP was measured across the whole of the IO curve.

Second, it aimed to test the hypothesis that if a physiology/learning correlation could be found in the hippocampus, it would be specific to spatial learning and dissociable from non-spatial learning. Therefore, spatial training was conducted prior to LTP induction, and after the completion of spatial training and LTP phases a final phase was instigated in which animals were trained on a non-spatial task, a tone-click discrimination. The timing of this phase with respect to tetanisation also permitted investigation of the possibility that tetanisation might facilitate simple conditioning in rats, as it appears to do in rabbits (Berger, 1984).

5.2 Experiment 4 Methods

The time course of this experiment is shown in Fig. 5.2.

HF	surgery + recovery	watermaze pretraining	baseline 5 days	watermaze training	tetanisation 8 d + Sk. box pretraining last 3 days	Skinner box training
LF	surgery + recovery	watermaze pretraining	baseline 5 days	watermaze training	low freq. 8 d + Sk. box pretraining last 3 days	Skinner box training
UC		watermaze pretraining	handling 5 days	watermaze training	handling 8 d + Sk. box pretraining last 3 days	Skinner box training

Figure 5.2 Design of Experiment 4.

5.2.1 Surgery

Twenty-seven male Lister hooded rats (250-400 g) were implanted with bilateral perforant path and dentate gyrus electrodes as described in the General Methods section. The stimulating electrodes for this experiment were monopolar, for ease of construction and because behavioural effects of current spread outside the perforant path were not in question in this experiment. Insulation was scraped from the tip to a distance of 0.5-1.0 mm. Electrophysiological testing began 2-6 weeks later. Thirteen rats served as unoperated controls.

5.2.2 Electrophysiology

Implanted rats with stable potentials in both hemispheres were assigned to the high-frequency group (HF, n = 16), and those in which the evoked potential deteriorated on one side because of shift of the recording electrode were assigned to a low-frequency

group (LF, n = 11) as described in the General methods. This group was included to control for effects of implantation and daily brain stimulation on the conditioning task. Because of unexpected changes to the evoked response following the low frequency stimulation protocol when begun on the day after spatial training, an additional 3 rats were included to test whether recent spatial training might interact with low frequency stimulation to induce potentiation. These animals had received LTP-inducing stimulation and spatial training several weeks previously and their LTP had decayed back to baseline.

Baseline and IO curve recording

Before training or tetanisation took place, 5 days of baseline evoked responses were collected. On the first day the stimulation intensity was set, as usual, to evoke a population spike of 1-3 mV. Each rat was acclimatised to the box for 20 min and then 10 low frequency pulses were collected from each hemisphere at 0.1 Hz followed by an IO curve. The 6th day was devoted to spatial training and no electrophysiological recording was conducted.

Tetanisation and low-frequency stimulation

Tetanisation or low-frequency stimulation began on day 7, the day after spatial training, and was administered daily according to HF protocol 1 for a period of 8 days in total. The low-frequency control stimulation consisted of replacement of each 10-pulse train with a single pulse (LF protocol 3, see Chapter 2). A post-stimulation baseline was recorded 20 min after the last train on each day. For the HF rats an IO curve was also recorded daily after the baseline.

5.2.5 Behaviour

Spatial training

Spatial training was conducted according to protocol 1 in the General Methods: namely, a single trial every 2 h to a total of 8 trials, followed by an absent-platform test 2 h later.

Conditioning

Simple conditioning (a tone-click discrimination for food reward) took place as described in the General Methods. Rats were run in pairs consisting of an experimental rat and a control wherever possible. A pilot study showed better learning when the CS+ was the click rather than the tone, and because the aim of this experiment was to correlate conditioning with both spatial learning and LTP, the click was therefore used as the CS+ (signalling reward) and the tone as the CS- (neutral with respect to reward) for all animals.

Pretraining in the Skinner boxes took place after each electrophysiology session on the 5th to the 7th days of tetanisation. Conditioning began after the last (8th) tetanisation session and lasted for 10 days. Again, rats were run in pairs. Conditioning was administered as 8 trials daily (four presentations of the CS+ and 4 of the CS-, pseudorandomly interspersed).

5.2.6 Histology

After the completion of the experiment, rats from the HF group underwent histological analysis as described in the General Methods.

5.3 Experiment 4 Results

One rat from each of the HF and LF groups showed a steady deterioration of evoked potentials across the tetanisation phase. Their data, both electrophysiological and behavioural, have therefore been excluded. There are thus 15 HF rats, 10 LF rats and 13 unoperated controls.

5.3.1 Behaviour

Spatial training

Acquisition of the watermaze task was measured by the latency to find the platform (Fig. 5.3A). The rats showed a steady decrease in time taken to find the platform across the 8 trials [$F(7,14) = 18.94$, $p < 0.0001$].

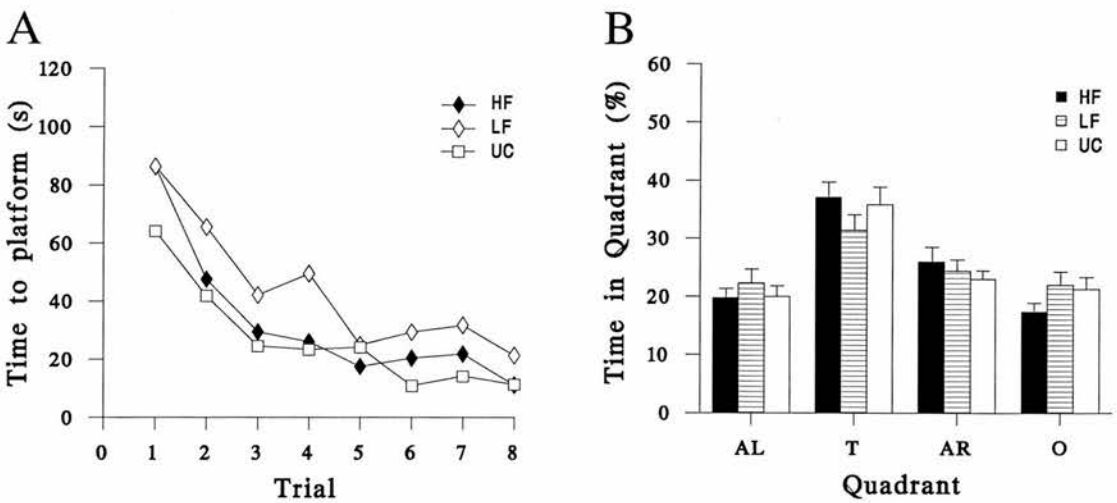


Figure 5.3 (A) Acquisition curves during watermaze training for HF, LF and UC groups. Watermaze training took place on the day prior to the start of tetanisation. Data points represent the group mean latencies to find the platform in each of the 8 trials. (B) Absent-platform test 2 h following the 8th trial. Values represent the group mean (± SEM) spent in each of the 4 quadrants of the pool while searching for the platform. AL = adjacent left, T = training, AR = adjacent right, O = opposite.

There was a group difference in watermaze acquisition [$F(2,35) = 3.85$, $p < 0.05$] due to the generally slower performance of the LF rats (Fig. 5.3A) but no interaction [$F(14,245) < 1$, NS]. By the final trial most rats were swimming directly to the platform. When the platform was removed from the pool all 3 groups spent significantly longer searching the quadrant where the platform had been [$F(3,6) = 22.53$, $p < 0.0001$; Fig. 5.3B]. The performance of each rat in the spatial task was expressed as the percentage of time spent searching in the training quadrant during the absent-platform test. There was no difference in performance between the 3 groups in performance on this test [$F(2,35) < 1$, NS].

Conditioning

Acquisition of the tone-click discrimination as measured by the increase in the elevation ratio is shown in Fig. 5.4. All 3 groups showed a significant increase across the 10 sessions [$F(9,234) = 6.49$, $p < 0.001$] and there was no difference between groups in the rate of acquisition [$F(2,35) < 1$, NS]. The overall performance of each rat in the task was expressed as the mean elevation ratio across all 10 sessions.

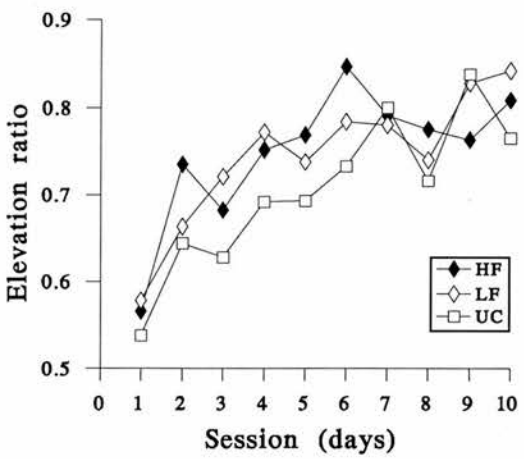


Figure 5.4 Acquisition curves during Skinner box training. Elevation ratio is calculated as the response rate during the CS+ (click) divided by the response during the CS+ and during the CS- (tone). The mean elevation ratio for all 10 trials was used as the index of conditioning ability for each rat.

Comparison of spatial with conditioning task

For all 38 rats there was no correlation between performance on the spatial task (time spent in the training quadrant on the absent-platform test) and performance in the Skinner box (mean elevation ratio; $r = -0.06$, NS; Fig. 5.5)

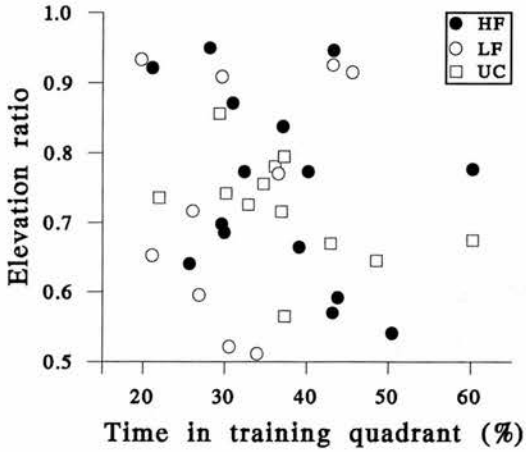


Figure 5.5 Correlation of performance in the watermaze with performance in the Skinner box. There was no correlation between ability on the two tasks.

5.3.2 Electrophysiology

LTP analysis

The evoked potentials before and after HF or LF stimulation are shown in Fig. 5.6.

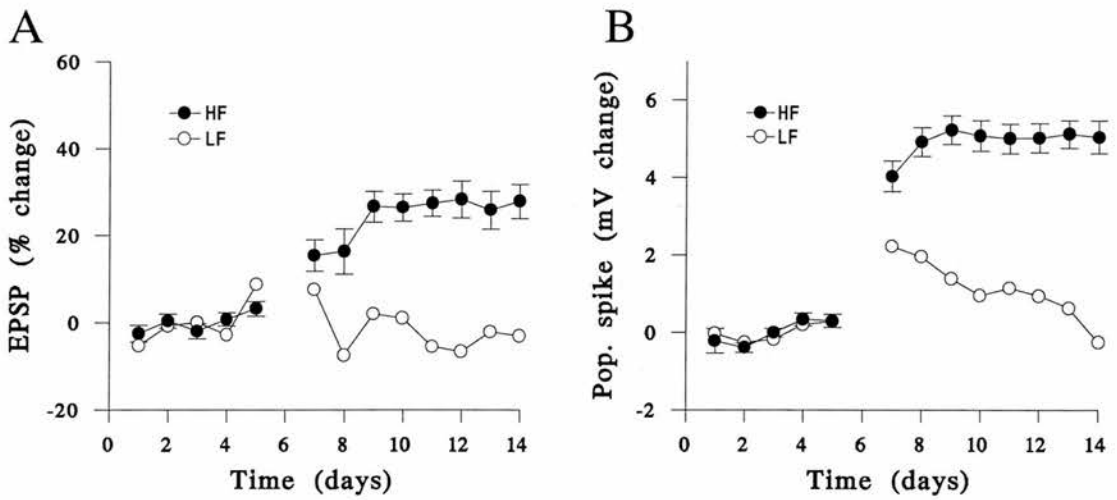


Figure 5.6 Change in evoked potentials following 8 days of tetanisation (HF group) or low-frequency stimulation (LF group). A plateau appears to have been reached by the tetanised rats on the third day for both EPSP and population spike. Note the large increase in the population spike following the LF stimulation protocol, which became less with each successive day of stimulation.

Analysis of variance of the evoked potentials in the HF and LF groups during the baseline and LTP induction phases revealed a significant interaction between groups and phases reflecting the increase in the HF group, following tetanisation, in both the EPSP [$F(1,23) = 26.65$, $p < 0.0001$] and the population spike [$F(1,23) = 56.88$ $p < 0.0001$]. Following low-frequency stimulation, although there was no change in the EPSP, the population spike showed a striking increase which declined with successive stimulation sessions. A two-tailed paired *t*-test comparing the baseline with the first post-stimulation values

confirmed that the EPSP showed no significant change (t = -1.06, NS) while the change for the population spike was highly significant (t = -3.62, $p < 0.01$). In the three extra animals which received low frequency stimulation after having been trained sometime previously, a population spike increase was also noted (data not shown).

Correlation

There was no correlation between spatial learning performance and LTP of the EPSP or population spike at either day 5 or day 8 of tetanisation (r's all < 0.41, NS; Fig. 5.7). Similarly, there was no correlation of either parameter with performance on the conditioning task (r's all < 0.45, NS; Fig. 5.8).

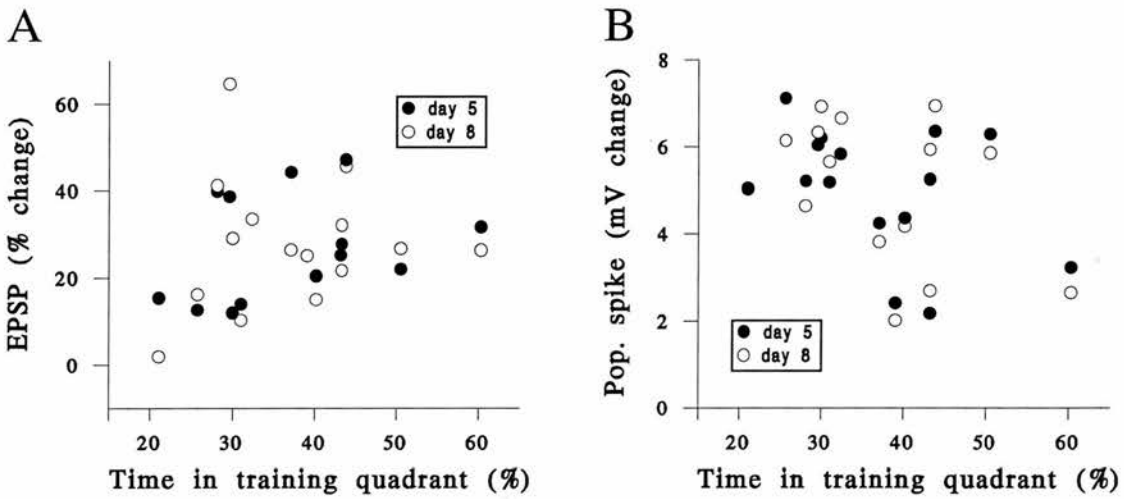


Figure 5.7 Comparison of spatial learning performance with subsequent cumulative level of LTP on the 5th and 8th days of tetanisation revealed no significant correlation for either the EPSP (A) or the population spike (B).

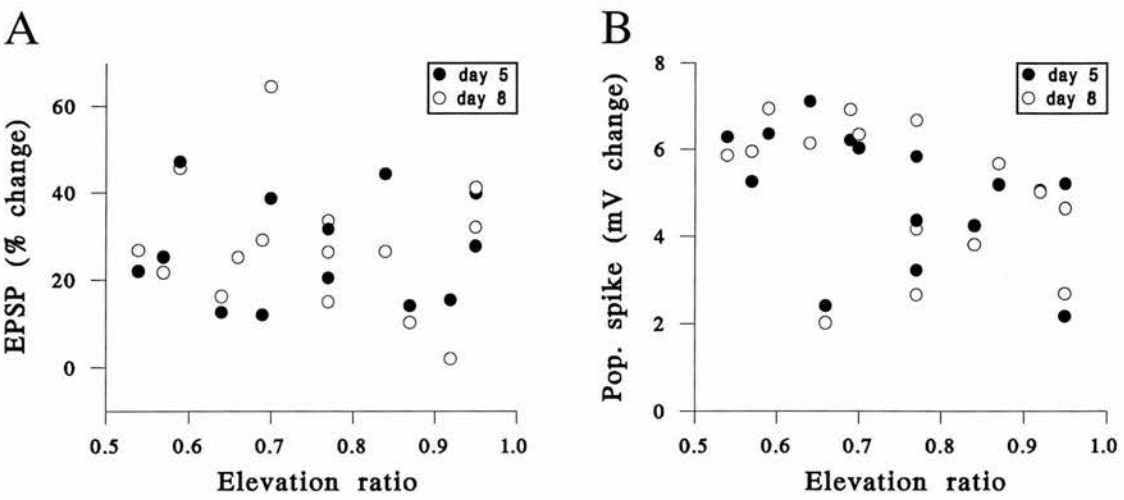


Figure 5.8 Comparison of Skinner performance (mean elevation ratio over all 10 sessions) with subsequent cumulative level of LTP on the 5th and 8th days of tetanisation revealed no significant correlation for either the EPSP (A) or the population spike (B).

IO curve analysis

The raw values for the evoked potentials were summed across the 20 stimulus intensities, yielding a value effectively representing the area under the curve. The values of these areas before and after tetanisation are shown in Table 5.1.

	Stimulus intensity (μA)	Baseline EPSP (Σ_0^{20} mV/ms)	Baseline spike (Σ_0^{20} mV)	Final EPSP (Σ_0^{20} mV/ms)	Final spike (Σ_0^{20} mV)	Training qdrnt time (%)	Elevation ratio
All HF rats	326	79.84	89.71	90.22	133.00	37.0	0.75
(n = 15)	(25)	(7.05)	(9.78)	(7.12)	(7.35)	(2.7)	(0.04)
poor WM	297	79.51	95.77	87.76	138.49	28.3	0.79
(n = 7)	(38)	(11.45)	(17.54)	(11.21)	(12.28)	(1.4)	(0.05)
good WM	352	80.13	84.40	92.36	128.19	44.7	0.71
(n = 8)	(34)	(9.38)	(10.91)	(9.72)	(9.07)	(2.6)	(0.05)
poor Sk. bx	345	70.07	78.85	78.17	129.76	37.4	0.63
(n = 7)	(30)	(11.27)	(12.52)	(9.49)	(11.34)	(3.5)	(0.02)
good Sk. bx	310	88.39	99.21	100.76	135.82	36.7	0.86
(n = 8)	(41)	(8.29)	(14.60)	(9.41)	(10.18)	(4.2)	(0.03)

Table 5.1 Mean raw values (± SEM) for stimulus intensity, EPSP and population spike before and after 5 days of tetanisation for the tetanised rats in Experiment 4. EPSP and population spike values were obtained by summing the values obtained at each of the 20 stimulation intensities, and thus effectively describe the area under the IO curve. Rats are divided according to both spatial and non-spatial learning ability. Also shown is the measure of spatial learning ability (time spent in training quadrant) and performance on the Skinner box task (elevation ratio). Note that the area under the post-tetanisation IO curves did not differ between good and poor learners. Note also that there appears to be no correlation between ability on the spatial task and ability on the Skinner box task.

One-tailed paired *t*-tests revealed a significant change in the area under the curve both for the EPSP ($t = -43.29$, $p < 0.0001$) and for the population spike ($t = -4.77$, $p < 0.001$). Analysis of variance comparing the good and poor watermaze learners before and after tetanisation revealed no difference in LTP for either the EPSP [$F(1,13 < 1$, NS] or population spike [$F(1,13) < 1$, NS]. Similarly, there was no difference for the good and poor Skinner box learners for the EPSP [$F(1,13) = 2.97$, NS] or population spike [$F(1,13) = 3.26$, NS].

The IO curves were then normalised to a baseline maximum of 100% as described in the General Methods. The curves before and after tetanisation for all 15 HF rats are shown in Fig. 5.9. Repeated-measures analysis of variance across the IO curve comparing pre-tetanisation with post-tetanisation values showed a significant group effect, reflecting the induction of LTP [$F(1,28) = 14.48$, $p < 0.001$ for the EPSP; $F(1,28) = 62.58$, $P < 0.0001$ for the spike]. There was also a significant interaction, illustrating a change in shape of the second curve with respect to the first [$F(19,532) = 2.32$, $p < 0.01$ for the EPSP; $F(19,532) = 9.62$, $p < 0.0001$ for the spike].

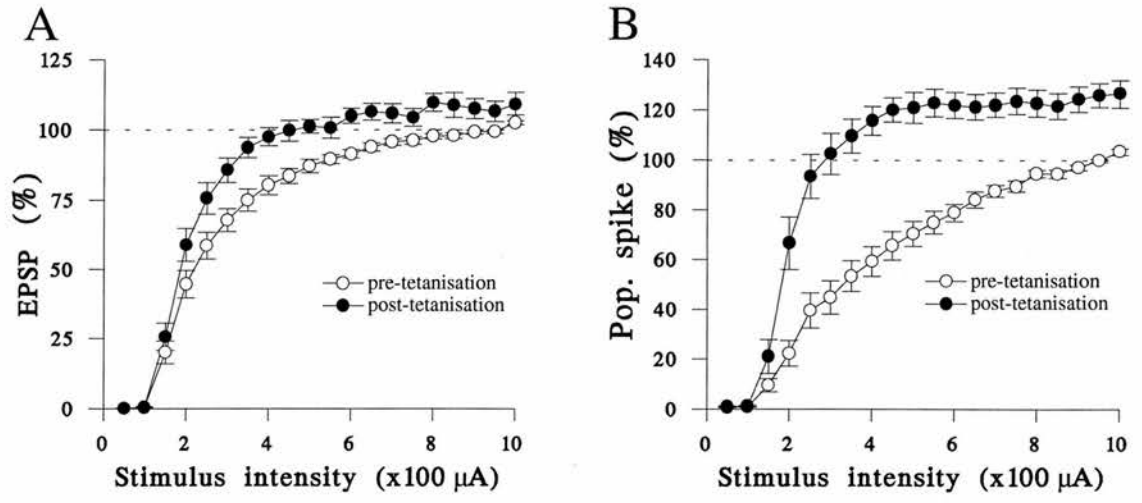


Figure 5.9 Normalised IO curves for the EPSP (A) and population spike (B) for all 15 tetanised rats before and after 5 days of daily tetanisation. Pre-tetanisation curves represent the mean of 5 days of baseline stimulation, scaled to a maximum of 100%. Post-tetanisation curves are expressed as a percentage of the maximum baseline value (shown by the dotted line). Population spike potentiation was relatively larger than for the EPSP, illustrating E-S potentiation. Note the convergence of the two curves in each graph as stimulus intensity increases.

The change in shape of the post-tetanisation curve was due to its convergence towards the baseline curve with increasing stimulus intensity, suggesting that LTP declined as test current strength was increased. A plot of percent potentiation against stimulus intensity confirmed that LTP declined exponentially as the current increased (Fig. 5.10).

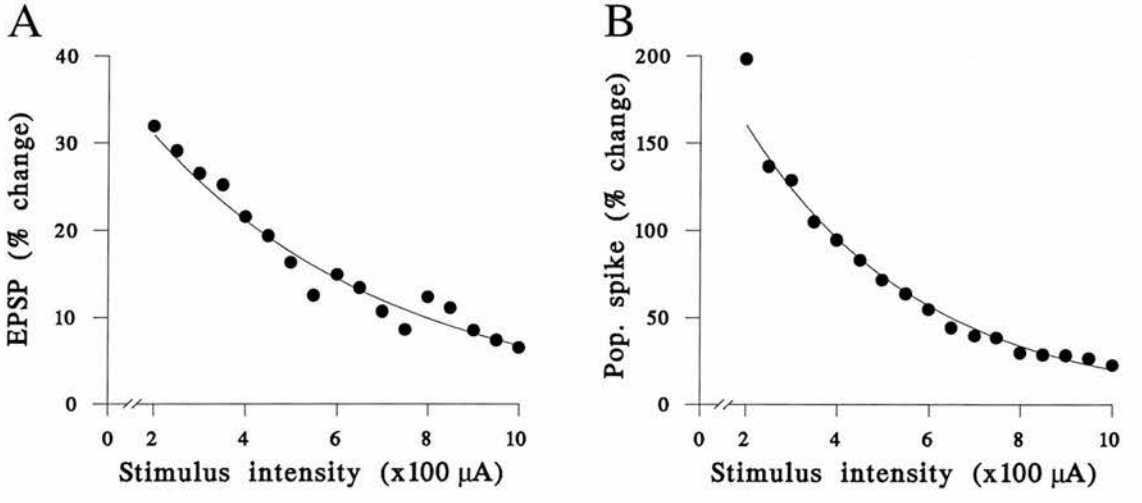


Figure 5.10 Exponential decline of LTP of the EPSP (A) and population spike (B) with increasing test stimulus intensity. The first 3 points (intensities 50-100 μA) have been omitted because of their extreme variability.

Exponential curves were fitted to the EPSP and spike data from individual rats according to the equation $y = Be^{-kt}$, yielding rate constant (k) and y-intercept (B) values. The log values of the y-intercept correlated with the rate constant for both EPSP ($r = -0.82$, $p < 0.001$) and the population spike ($r = -0.67$, $p < 0.01$; Fig. 5.11)

suggesting that the underlying process controlling the rate of convergence of the post-tetanisation IO curve to the baseline curve was related to the magnitude of LTP.

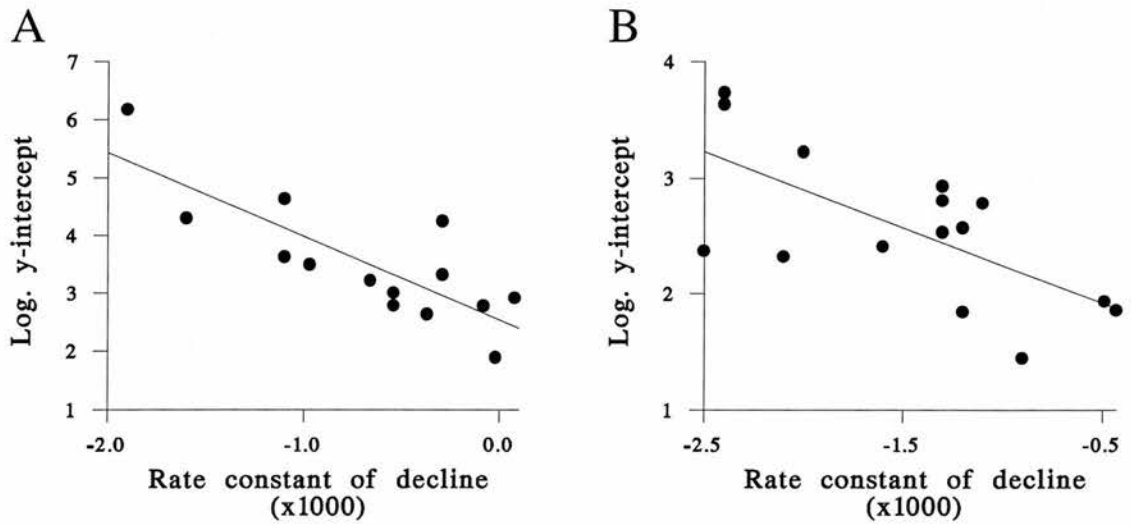


Figure 5.11 Fitting of exponential curves to the LTP decline data yielded a rate constant and *y*-intercept value for each rat. These two parameters correlated with each other.

5.3.3 Comparison of IO curve electrophysiology with behaviour

Animals were divided by the group means into good and poor spatial learners and good and poor Skinner box learners. Acquisition and absent-platform test performance for the watermaze task are shown in Fig. 5.12 for these two groups, and examples of the search path of a good and poor learner are shown in Fig. 5.13. There was no difference in acquisition between the two groups [$F(7,91) = 0.56$, NS]. The good learners spent significantly more time searching the training quadrant during the absent-platform test than the poor learners [$F(3,39) = 7.78$, $p < 0.001$].

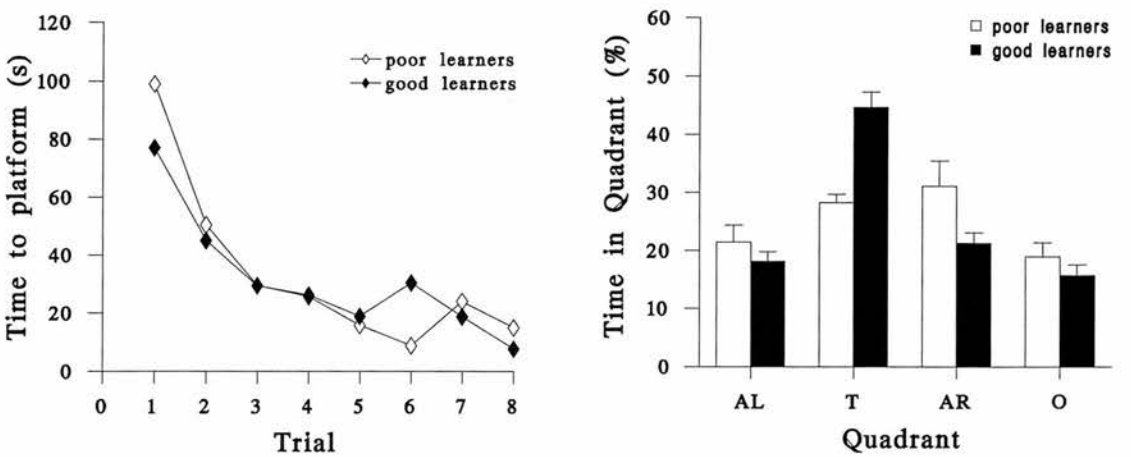


Figure 5.12 (A) Acquisition curves for the watermaze task for rats divided into good and poor learning groups on the basis of their training quadrant search times. There was no difference in latencies for the two groups. (B) Rats spending below the mean amount of time searching the training quadrant ("poor learners") spent significantly less time in this quadrant than rats searching for above the mean time ("good learners"). AL = adjacent left, T = training, AR = adjacent right, O = opposite.

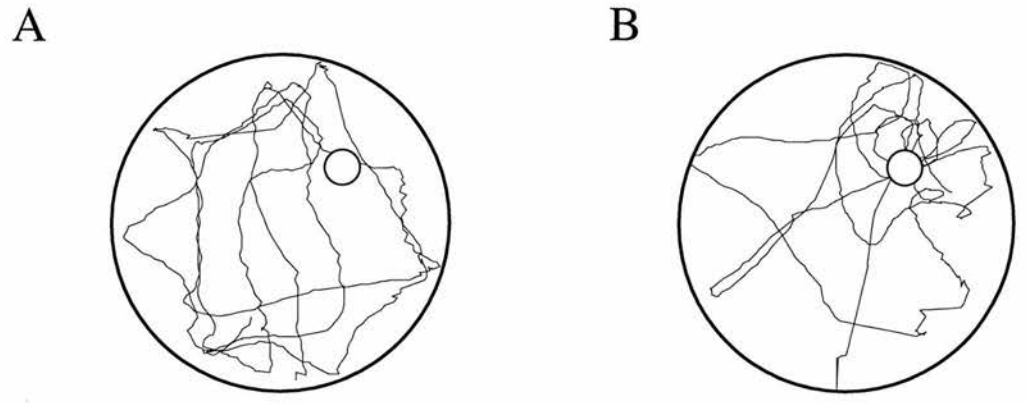


Figure 5.13 Example of the search path of a poor learner (A) and a good learner (B) during the absent-platform test. The platform had been located in the upper right-hand quadrant of the pool during the training trials.

Comparison of electrophysiology with behaviour will be described first for animals grouped according to watermaze performance, and then grouped according to Skinner box performance.

Comparison of IO curve electrophysiology with spatial learning

Raw baseline IO curves for rats divided according to ability in the watermaze are shown in Fig. 5.14. There was no difference in the magnitude of the baseline responses for either the EPSP or population spike [$F(1,13) < 1$, NS for both]. Similarly, analysis of the interaction revealed no difference in the shape of the baseline curves [$F(19,247) < 1$, NS for both].

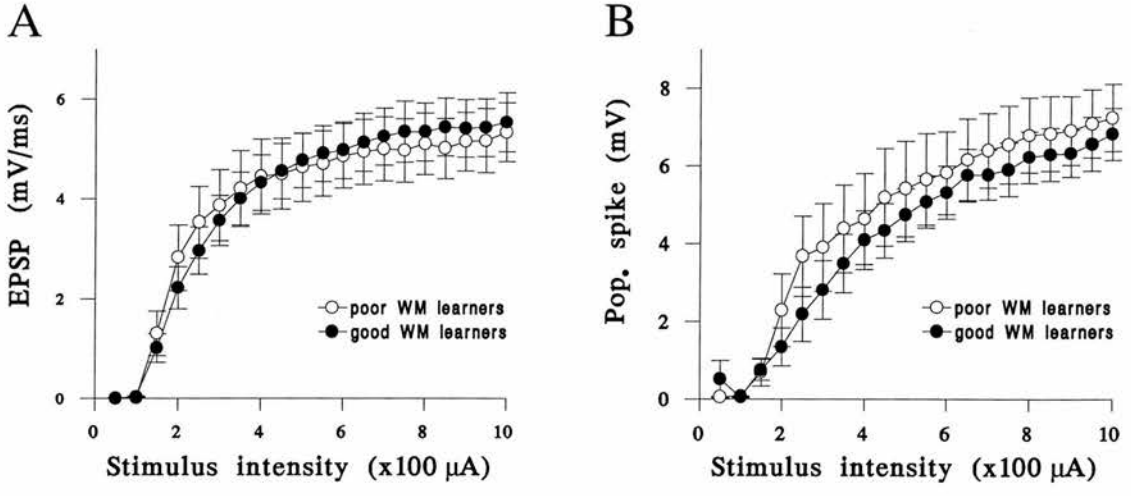


Figure 5.14 Raw pre-tetanisation IO curves for EPSP (A) and population spike (B) showing no baseline difference between rats which were poor (n = 7) or good (n = 8) water maze learners. Each curve is the group mean (± SEM) of 5 days of baseline recordings.

IO curve potentiation was then examined for the poor and good learning groups, respectively. The normalised IO curves for the EPSP before and after tetanisation compared for the poor learners are shown in Fig. 5.15A.

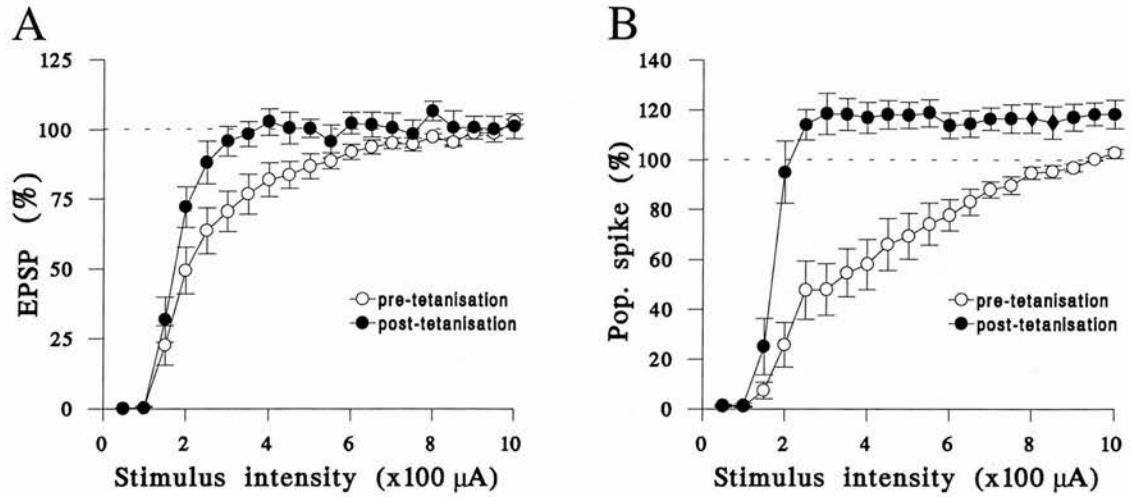


Figure 5.15 Normalised IO curves for EPSP (A) and population spike (B) before and after tetanisation for the poor spatial learners. There was substantial LTP at the low end of the curve but little or none at the high test intensities.

Comparison of the post-tetanisation curve with the pre-tetanisation curve revealed no significant group effect for the EPSP, suggesting that little LTP was induced in this group [$F(1,12) = 4.34$, NS]. The population spike, however, showed a significant change [$F(1,12) = 30.13$, $p < 0.0001$; Fig. 5.15B]. There was also a significant interaction for both EPSP [$F(19,228) = 3.33$, $p < 0.0001$] and population spike [$F(19,228) = 10.49$, $p < 0.0001$], reflecting a change in the shape of the post-tetanisation curves for both parameters.

The good learners demonstrated a significant increase in the magnitude of the post-tetanisation curves (Fig. 5.16) for both EPSP [$F(1,14) = 10.30$, $p < 0.01$] and population spike [$F(1,14) = 28.64$, $p < 0.0001$].

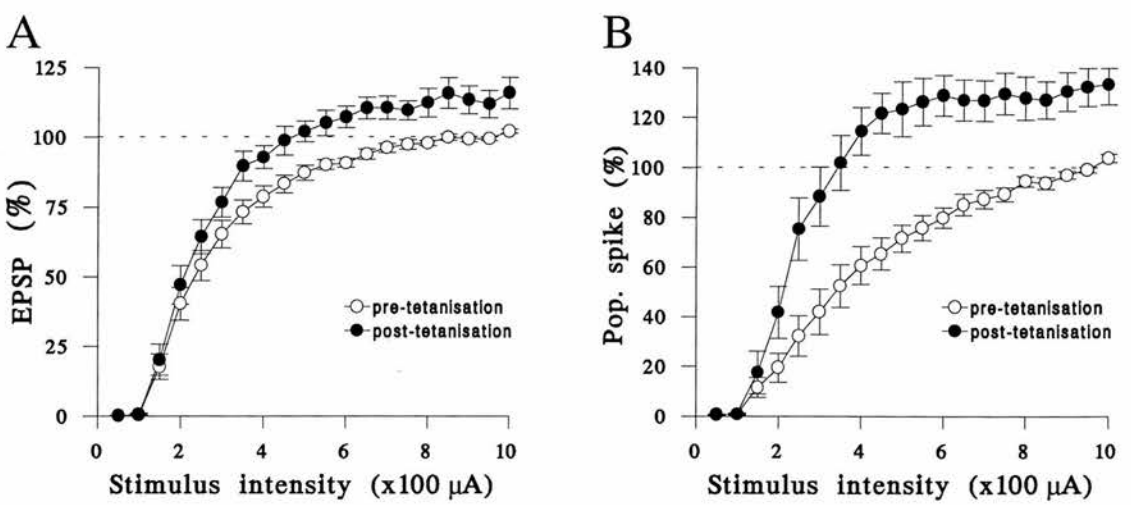


Figure 5.16 IO curves for the EPSP (A) and population spike (B) before and after tetanisation for the good spatial learners. There was moderate LTP across the whole extent of the IO curve.

There was no interaction for the EPSP [F(19,266) = 1.52, NS], suggesting that the post-tetanisation curve did not differ in shape from the pre-tetanisation curve. However, there was a significant interaction for the spike [F(19,266) = 4.67, $p < 0.0001$].

From the above analysis, it appeared that poor learners differed from good learners in two respects: first, while poor learners showed little EPSP potentiation, they showed a change in the post-tetanisation EPSP IO curve shape. Second, while good learners showed significant post-tetanisation potentiation, the EPSP curve did not differ in shape from the baseline. This observation prompted a comparison of the post-tetanisation curves for the two groups. The results are shown in Fig. 5.17. Analysis of variation of the two curves revealed a highly significant interaction, confirming that post-tetanisation curves differed in shape for both EPSP [F(19,247) = 5.92, $p < 0.0001$] and population spike [F(19,247) = 4.86, $p < 0.0001$]. Specifically, while poor learners showed little potentiation at high stimulus intensities, they showed greater LTP than the good learners at low stimulus intensities. This was apparent for both EPSP and population spike, ruling out a contribution from artefact due to the way in which the EPSP was measured.

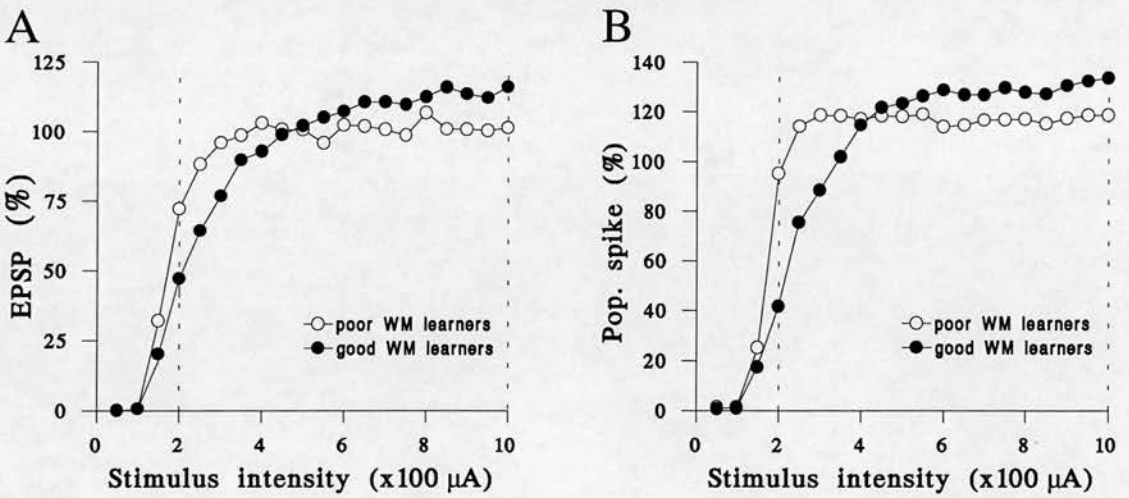


Figure 5.17 The post-tetanisation curves for good and poor learners from the previous figure have been replotted on the same graphs, to allow direct comparison of the curves. (A) EPSP, (B) population spike. Note the crossover of the curves after tetanisation. The dotted lines indicate the stimulus intensities at which within-animal comparisons were made with watermaze performance (next figure).

Post-tetanisation IO curves differed most between good and poor learners at two stimulus intensities: at 200 μA, where the poor learners showed the greatest LTP, and at 1000 μA where they showed the least. Accordingly, evoked potential size was compared between the two groups at these two stimulus intensities (Fig. 5.18). At 200 μA there was a significant negative correlation between spatial learning performance and size of the EPSP ($r = -0.60$, $p < 0.05$) and the population spike ($r = -0.63$, $p < 0.05$). At 1000 μA, by contrast, the correlation had reversed sign and there was a significant *positive* correlation

between spatial learning and EPSP size ($r = 0.64$, $p = 0.01$) and spike height ($r = 0.52$, $p < 0.05$).

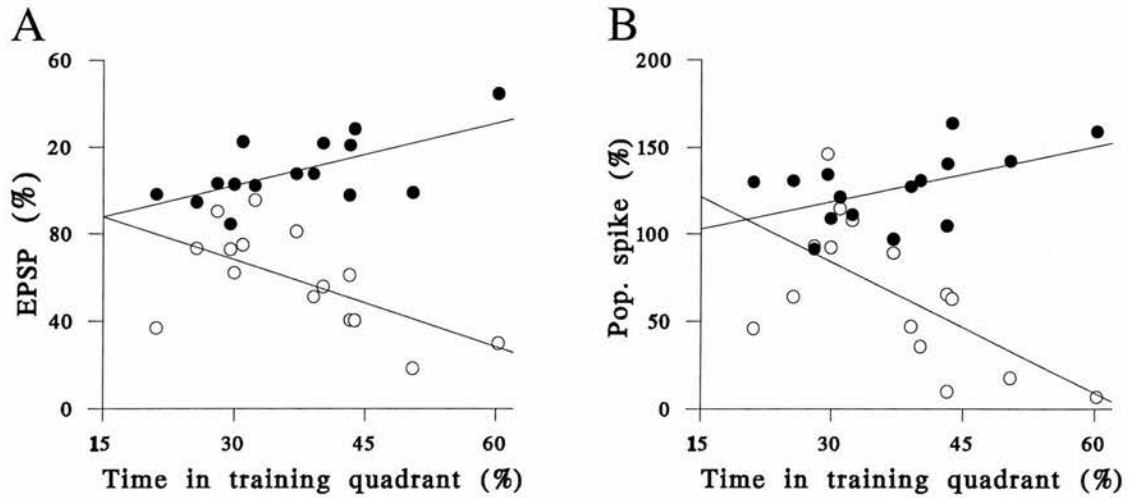


Figure 5.18 Correlation of individual spatial learning scores with the post-tetanisation evoked potentials measured at two points on the IO curve (shown by the dotted lines in the previous figure): low (200 μA, hollow circles) and high (1000 μA, filled circles) for the EPSP (A) and population spike (B).

Because of the above variable decline of potentiation across the IO curve, an invariant measure of LTP was sought for the purposes of obtaining a correlation with spatial learning which did not depend on stimulus intensity. Two obvious such parameters are the *y*-intercept and rate constant of the decline of LTP across the IO curve, obtained by the curve-fitting method described earlier. However, this procedure yielded somewhat variable results, and so the parameters for LTP to be used in the following analysis were obtained by first linearising the curves by means of a semi-log plot (EPSP) or log-log plot (spike), and then fitting a regression line to the points (Fig. 5.19).

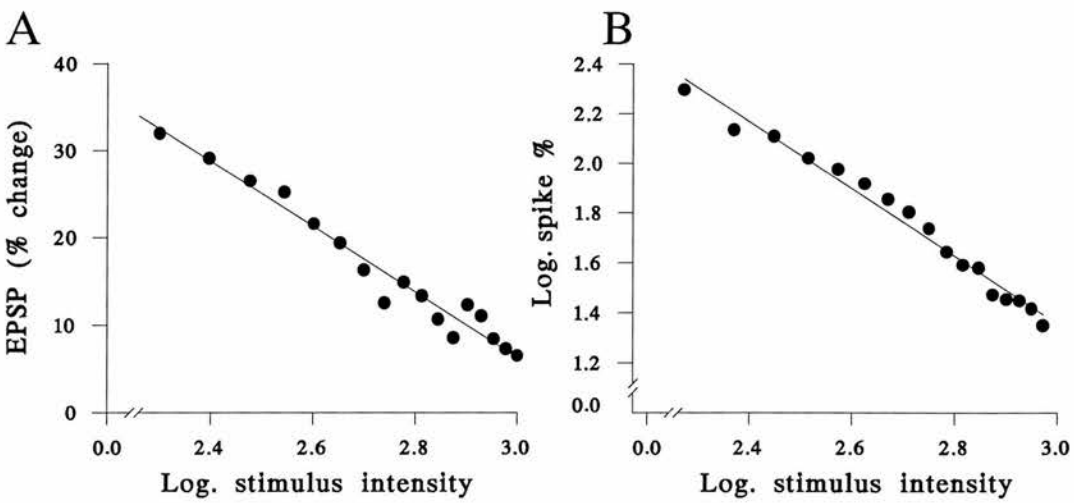


Figure 5.19 Linearisation of the LTP curves shown in Fig. 5.10 by mean of a semi-log plot (EPSP, A) or log-log plot (population spike, B). The *y*-intercept of the curve represents the hypothetical potentiation which would be recorded close to zero stimulus intensity. The gradient of the line describes the rate at which the post-tetanisation curve converges towards the pre-tetanisation curve.

This procedure yielded a gradient and a *y*-intercept for EPSP and population spike. The regression analysis was applied to the raw data from individual rats, yielding a *y*-intercept and gradient value for each animal. These two parameters correlated very highly with each other, reflecting the exponential nature of the LTP decline. They also correlated significantly with spatial learning ability (Fig. 5.20). The correlation for the *y*-intercept measure was negative for both EPSP ($r = -0.63$, $p < 0.05$) and the population spike ($r = -0.63$, $p < 0.05$). The correlation for the gradient measure was positive for both EPSP ($r = 0.67$, $p < 0.01$) and population spike ($r = 0.65$, $p < 0.01$). In other words, rats with poor spatial learning ability showed a greater *y*-intercept (*i.e.* theoretical potentiation at near-zero stimulus intensity) but a faster decline across the IO curve.

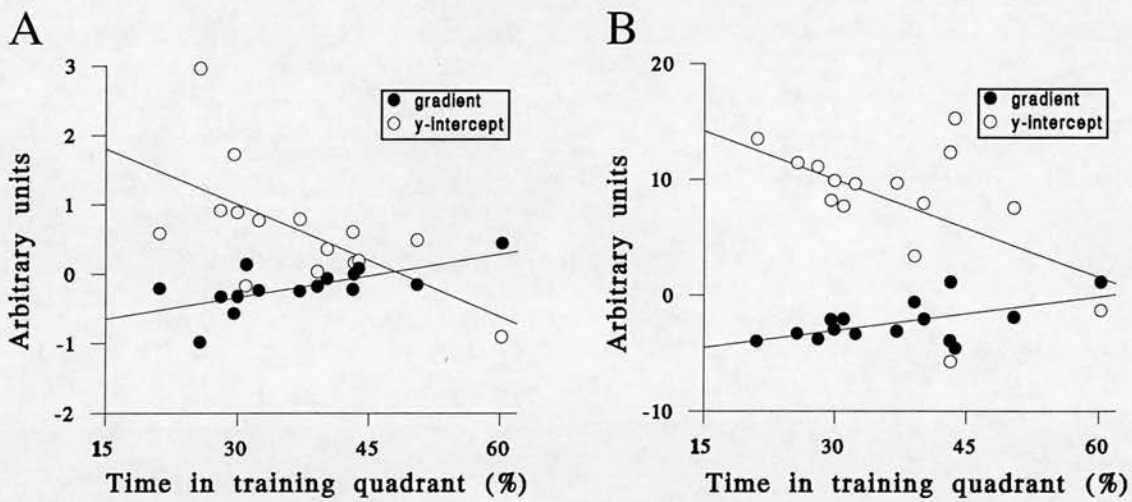


Figure 5.20 The two parameters describing the change of potentiation across the IO curve: that is, the *y*-intercept and the gradient of the linear regression line, were plotted against spatial learning ability for the EPSP (A) and population spike (B). There was a significant correlation of each of the parameters with learning for both the EPSP and the population spike.

To test the possibility that the differential decline of LTP across the IO curve between the two groups was due to post-tetanisation differences in feedforward inhibition, E-S curves were plotted for the two groups (Fig. 5.21). A difference in feedforward inhibition, as discussed in Chapter 1, would be expected to show up as a difference in post-tetanisation E-S slope. Poor learners showed a flattening of the post-tetanisation E-S slope but this difference was very slight (Fig. 5.21B).

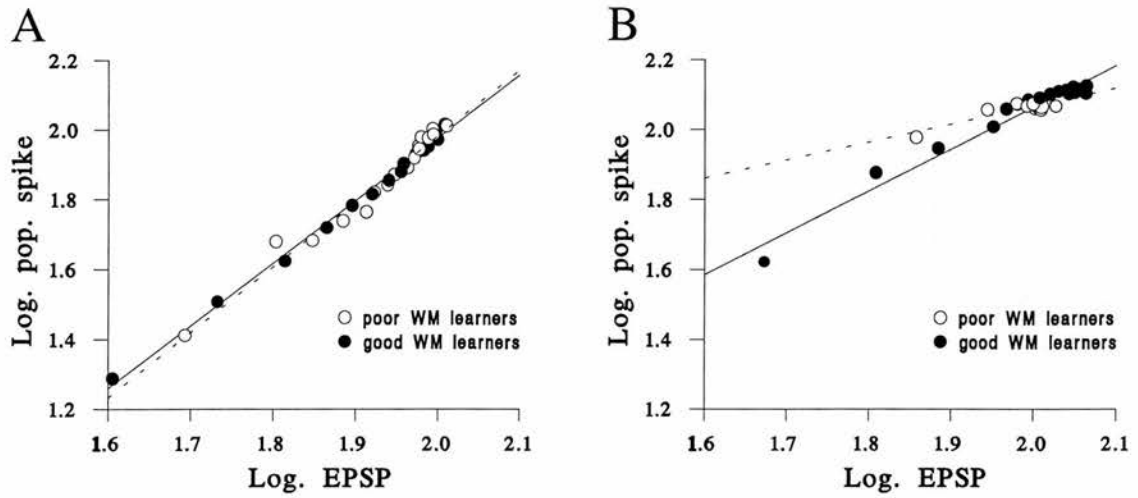


Figure 5.21 E-S curves before (A) and after (B) tetanisation for good and poor spatial learners. There was no difference between good and poor learners either in the pre- or the post-tetanisation E-S relationship.

Finally, the time course of the development of the disparity between LTP in the two groups was plotted (Fig. 5.22). This was done by plotting the evoked potential size at 200 μA and 1000 μA on each of the 5 tetanisation days. Although there was a trend towards greater LTP in the poor learners at 200 μA and less at 1000 μA on the first two days, the evoked potentials did not clearly separate until the third day and reached maximum disparity on the 4th day.

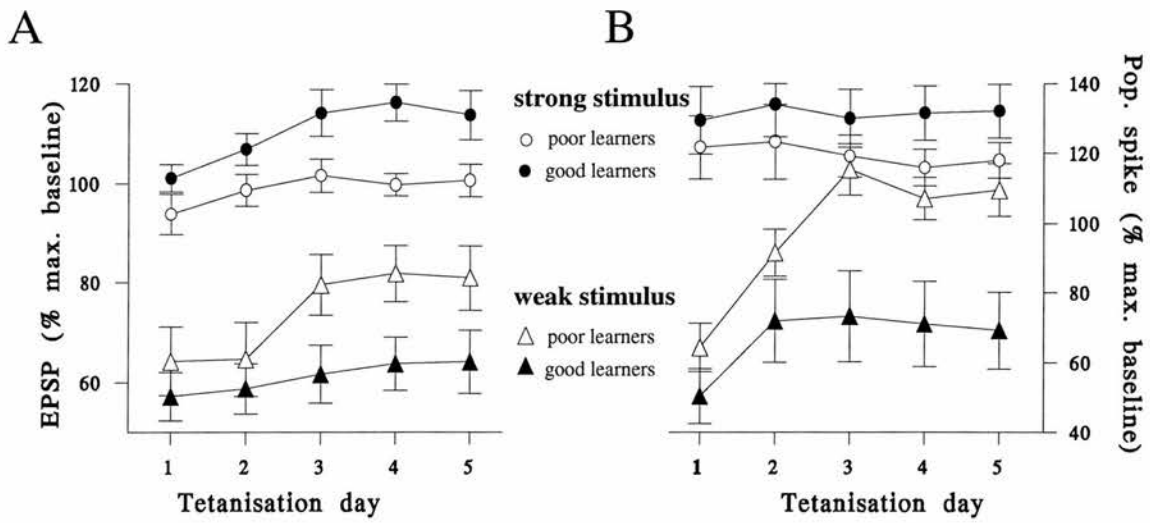


Figure 5.22 Time course of the development of the separation of LTP between good and learners for the EPSP (A) and population spike (B) over the 5 days of tetanisation. Circles represent the evoked response measured using a strong stimulus (900-1000 μA) and triangles represent the response measured using a weak stimulus (200-300 μA). The separation became maximal after 3-5 days of daily tetanisation. Note that the relationship reversed between weak and strong stimulus intensities.

The results of the spatial learning/electrophysiology comparison may be summarised as follows: while poor spatial learners showed less LTP (consistent with the findings presented in Chapter 3) when evoked potentials were measured using strong test pulses,

they showed *greater* LTP when measured using weak test pulses, resulting in a reversal of the sign of the correlation between these two stimulus intensities. This occurred because of a decline of LTP across the IO curve which differed between the two groups. While poor learners showed high levels of LTP when measured with test pulses close to zero intensity (a measure estimated by backwards-extrapolating the linear regressions depicted in Fig. 5.19), their LTP declined more rapidly than in the good learners, so that by the middle of the curve the two groups were showing approximately equal levels of LTP and by the top end of the curve, the good learners were showing greater LTP than the poor learners. A curve-fitting procedure which estimated the rate constant of the LTP decline confirmed a within-animal correlation between the rate of decline and LTP magnitude (Fig. 5.12). Because of the correlation between LTP magnitude and its rate of decline, either parameter provides a measure of LTP which is invariant with respect to stimulus intensity. When such a measure is used, there is a significant correlation of LTP with spatial learning ability (Fig. 5.20).

Comparison of IO curve electrophysiology with conditioning

The above analyses were repeated for the same rats divided according to their performance on the Skinner box task. Analysis of the 2-trial blocks confirmed that the poor learners performed significantly worse than the good learners [$F(4,52) = 4.61$, $p < 0.01$]. The raw baseline curves did not differ in magnitude or shape between good and poor Skinner box learners for either EPSP or population spike [F's all < 2, NS].

Comparison of the pre- and post-tetanisation curves is shown in Fig. 5.23 for the poor learners.

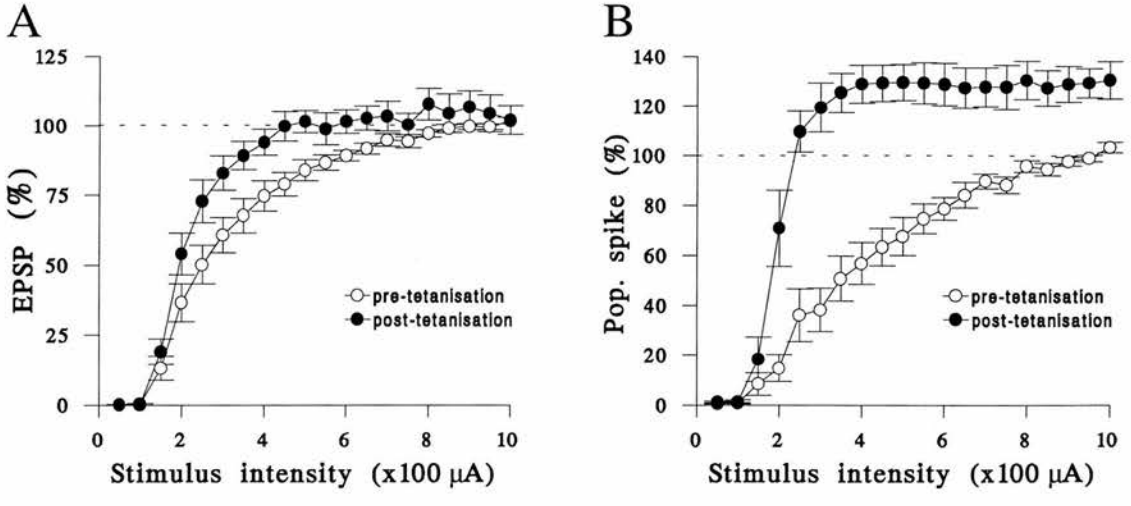


Figure 5.23 IO curves before and after tetanisation for poor Skinner box learners for EPSP (A) and population spike (B). There is a similar pattern as for the good and poor spatial learners, with the post-tetanisation curve showing a steep early phase and a sudden plateau.

There was a significant increase in IO curve magnitude for both EPSP [$F(1,12) = 6.99$, $p < 0.05$] and population spike [$F(1,12) = 45.26$, $p < 0.0001$], reflecting the induction of LTP. There was also a significant interaction for both EPSP [$F(19,228) = 2.26$, $p < 0.01$] and population spike [$F(19,228 = 10.13$, $p < 0.0001$], illustrating the change in post-tetanisation curve shape.

The good learners also showed significant IO curve LTP [$F(1,14) = 8.36$, $p < 0.05$ for the EPSP; $F(1,14) = 24.73$, $p < 0.001$ for the spike; Fig. 5.24]. The interaction was not significant for the EPSP [$F(19,266) < 1$, NS] but was for the spike [$F(19,166) = 2.31$, $p < 0.01$].

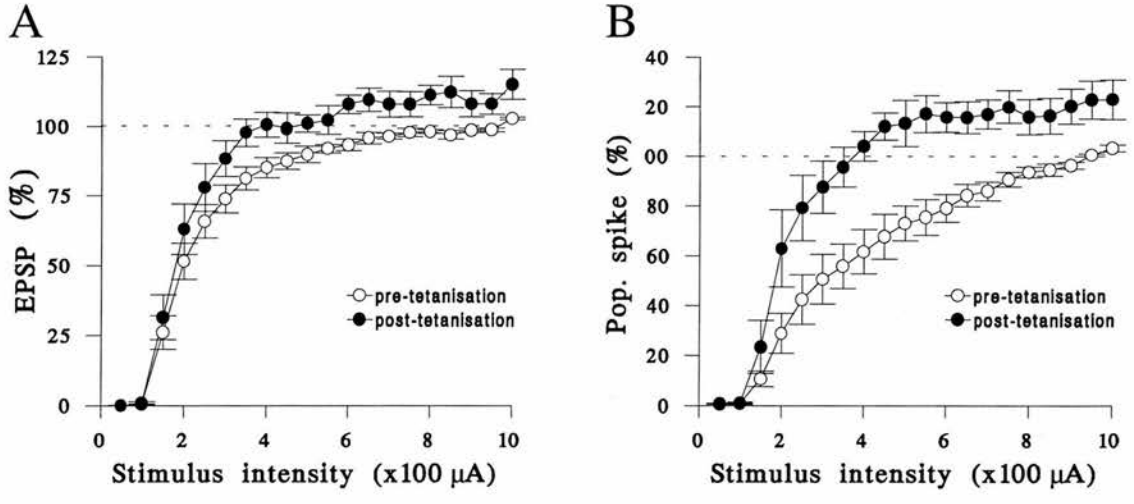


Figure 5.24 IO curves before and after tetanisation for the good Skinner box learners for EPSP (A) and population spike (B). The pattern of convergence of the pre- and post-tetanisation curves resembles that seen when the animals were divided according to spatial learning ability, with a more gradual post-tetanisation rise and a higher plateau.

Comparison of the post-tetanisation curves for the two groups (Fig. 5.25) revealed no significant interaction for either EPSP or population spike [F's < 1.2, NS].

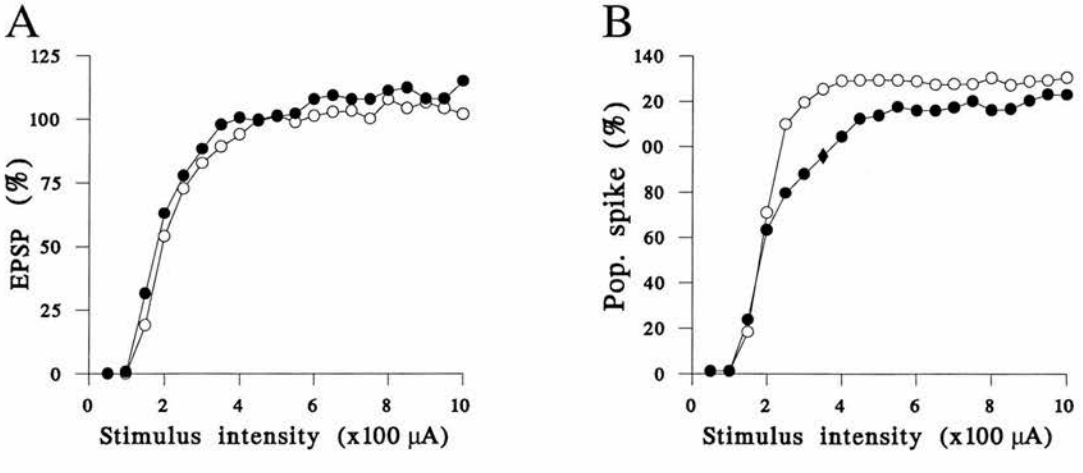


Figure 5.25 Post-tetanisation IO curves compared between good (filled circles) and poor (hollow circles) Skinner box learners. The crossover seen when the rats are divided according to spatial ability is not seen. However, spike potentiation appears to be increased in the poor learners, though this was not significant.

In support of the IO curve findings, there was no correlation of evoked potential size at 200 μA or 1000 μA with performance on the Skinner box task (r's all < 0.30, NS; Fig. 5.26). In addition, neither the gradient nor the y-intercept of the LTP decline correlated with performance on the Skinner box task (r's all < 0.35, NS).

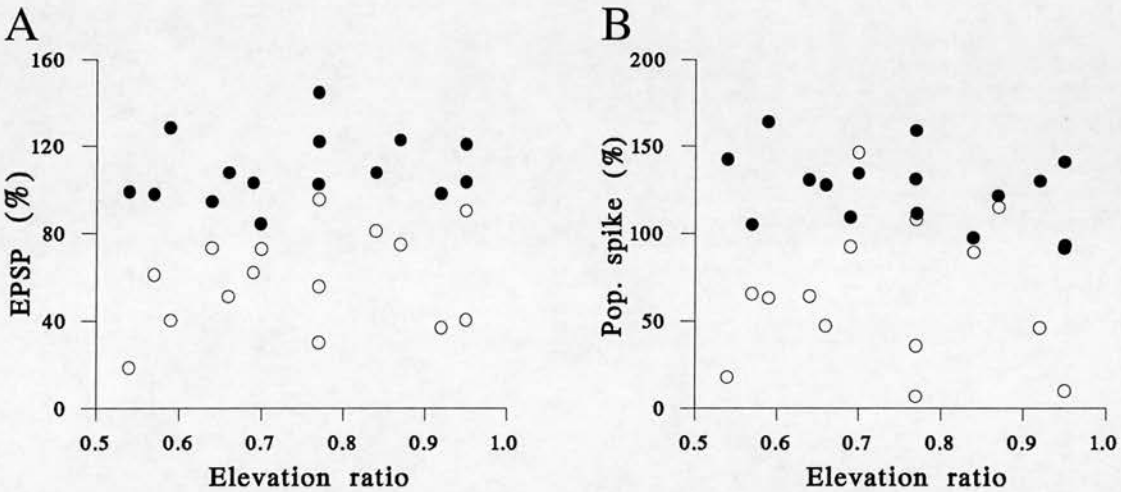


Figure 5.26 There was no correlation of performance on the discrimination task (mean elevation ratio over all 10 sessions) and potentiation of either EPSP (A) or population spike (B) at low or high stimulus intensities (*cf* Fig. 5.18).

Good versus poor overall learners

Although there was no correlation between IO curve potentiation and Skinner box learning, the graphs in Figs. 5.23 and 5.24 suggest a trend towards a similar relationship between pre- and post-tetanisation IO curves as was seen in the good and poor watermaze learners: that is, a convergence of the curves in the poor learners but not in the good learners. More detailed analysis suggested that the trend was mainly contributed by a subset of rats which were good and poor at both tasks: that is, the rats showing the greatest and the least convergence of the IO curves were poor (n = 3) and good (n = 4), respectively, at both spatial and Skinner box learning. Accordingly, the correlational analysis was repeated only for these rats (Fig. 5.27).

When slope and y-intercept measures were plotted against spatial learning for these animals, the correlation increased markedly for both slope ($r = 0.93$, $p < 0.0001$ for the EPSP and $r = 0.83$, $p < 0.01$ for the spike) and y-intercept ($r = -0.90$, $p < 0.0001$ for the EPSP and $r = -0.74$, $p < 0.05$). The correlation also improved for comparison with Skinner box performance, although it still did not reach significance (data not shown).

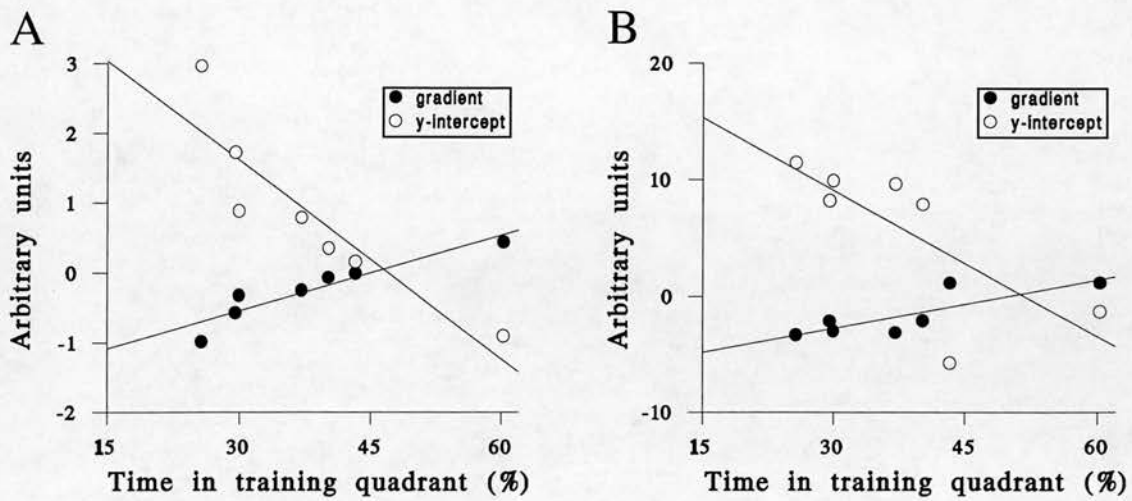


Figure 5.27 When the correlation was repeated for those rats scoring consistently well or poorly on both the spatial and non-spatial task the correlation increased for both EPSP (A) and poipulation spike (B).

5.3.4 Histology

After completion of all experimental manipulations (including, for some rats, Experiment 5: see below) the 15 tetanised rats underwent histological analysis of the electrode sites, as described in the General Methods. This was to determine whether a systematic variation in electrode position could account for the distribution of LTP observed. In addition, the possibility that hippocampal damage associated with the electrodes could have accounted both for the spatial performance and pattern of potentiation seen in the poor learning rats was investigated. An example of a representative recording and stimulating electrode site is shown in Fig. 5.28. The distribution of electrode sites for all 15 rats is shown schematically in Fig. 5.29. In general, little tissue damage was seen around the recording electrode sites. In two rats from the poor learning group, some cavitation was seen in the angular bundle around the site of the stimulating electrode unilaterally. A third rat from this group showed some overlying cortical damage with gliosis around the electrode tip unilaterally. A rat from the good learning group also showed slight unilateral electrode-tip gliosis. Exclusion of these 4 rats from the correlational analysis did not abolish any of the correlations presented earlier. On the contrary, the correlations increased for all measures except the slope and *y*-intercept comparisons of population spike LTP with spatial learning, which decreased slightly. It thus appears unlikely that the results of the present experiment could be attributed to electrode-associated brain damage.

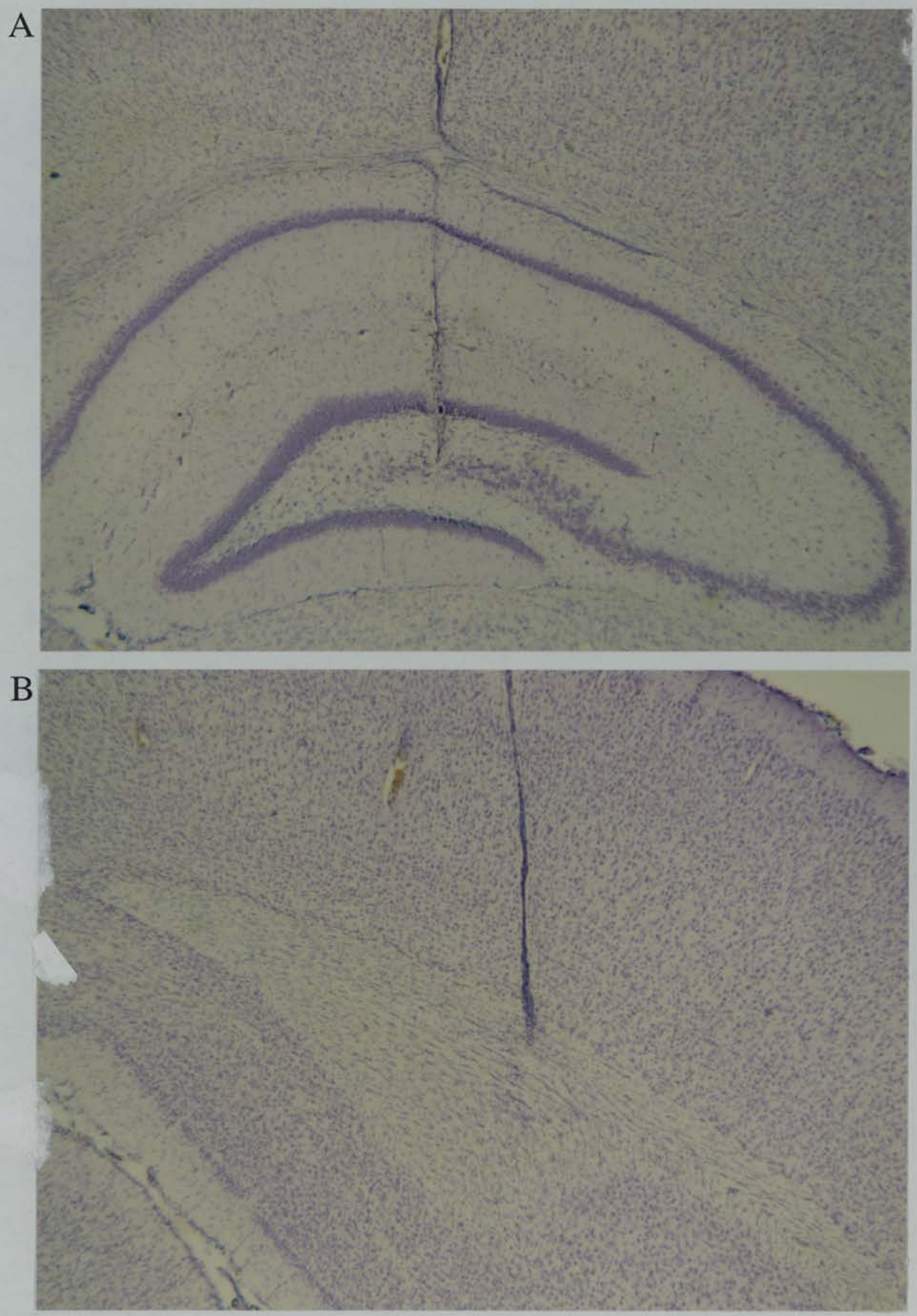


Figure 5.28 Photomicrograph of a representative recording (A) and stimulating (B) electrode site. The sections were stained with cresyl violet. Scale = 1:400

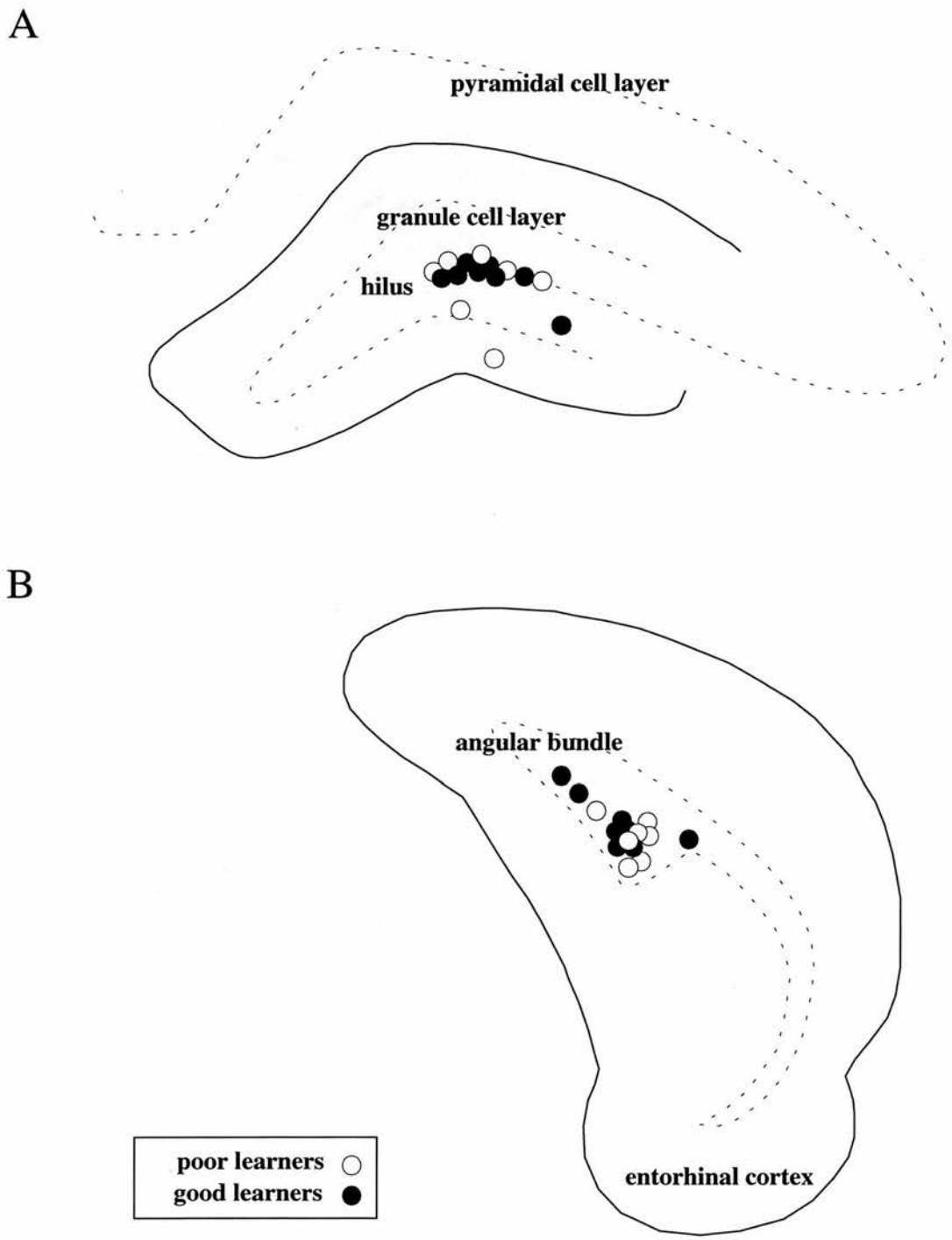


Figure 5.29 Distribution of electrode sites for the good and poor spatial learners. Because sites for the two hemispheres in individual rats were comparable, for clarity each point represents the mean location of both electrode tips for each rat. (A) recording electrodes. (B) stimulating electrodes.

5.4 Discussion of Experiment 4

The first major finding of Experiment 4 is that measured LTP varied in a systematic manner with increasing test pulse intensity. This result, as well as being interesting in its own right, may explain some of the contradictory findings emerging from the previous three experiments. The decline of LTP with increasing test stimulus intensity is discussed below, and a parameter for LTP estimation is derived which takes into account this variation across the IO curve.

The second major finding is that when a measure of LTP is used which is invariant with respect to stimulus intensity for a given animal, a correlation with learning becomes apparent, thus supporting the findings of Experiments 1 and 2. Experiment 4 produced the additional finding that this correlation appears, in reality, to be *negative:* that is, rats which are the best learners show the least amount of LTP. The implications of this unexpected finding for the plasticity/learning hypothesis are discussed in the next chapter.

5.4.1 Decline of LTP with test pulse intensity

One reason for recording whole IO curves in this experiment was to investigate the possibility that LTP might vary depending on the strength of the test pulses used (Cain *et al.,* 1993), thus possibly confounding quantitative LTP estimations with a measurement artefact and obscuring any LTP/learning correlations. Analysis of the post-stimulation IO curves for the tetanised rats in this experiment revealed that this was indeed the case: percent potentiation at each of the stimulus intensities decreased exponentially with increasing test current, at a rate that appeared to be faster for the population spike. However, it was found that the rate of decline varied considerably between animals, ranging from a very steep decline across the curve in some rats to a slight *increase* of potentiation in others. Furthermore, the rate of decline was found to correlate with the initial magnitude of LTP as would be measured at very small test currents (*i.e.* the *y*-intercept). This observation has not previously been reported. An explanation must address two questions: (1) why does LTP decline across the IO curve, and (2) why does this rate correlate with its magnitude?

Discussion of why the post-tetanisation curve converges towards its baseline with increasing test currents must centre on the reasons for the shape of the IO curve. Influences on the EPSP IO curve shape are as follows:

(1) Excitability distribution of perforant path fibres,

(2) Current spread and the limited size of the perforant path,

(3) Synaptic strength, and

(4) Non-linear summation of post-synaptic potentials.

Excitability distribution of perforant path fibres

Under conditions of constant synaptic strength, the excitability distribution of afferent fibres could influence the IO curve as follows. A small test pulse applied to the perforant path activates fibres possessing the lowest firing thresholds. As the current increases, an increasing proportion of fibres will cross their thresholds and begin generating action potentials. Assuming that the excitability distribution is Gaussian, the increase in evoked response size will assume a sigmoid shape as the local current intensity reaches and then exceeds the mean firing threshold of the fibres (Fig. 5.30A,C). A reduction in the overall mean firing threshold will shift the IO curve to the left, while a narrowing of the distribution will steepen it (Fig. 5.30B,D). It follows that one possible explanation for a change in IO curve shape after tetanisation could be that the fibre excitability profile changes.

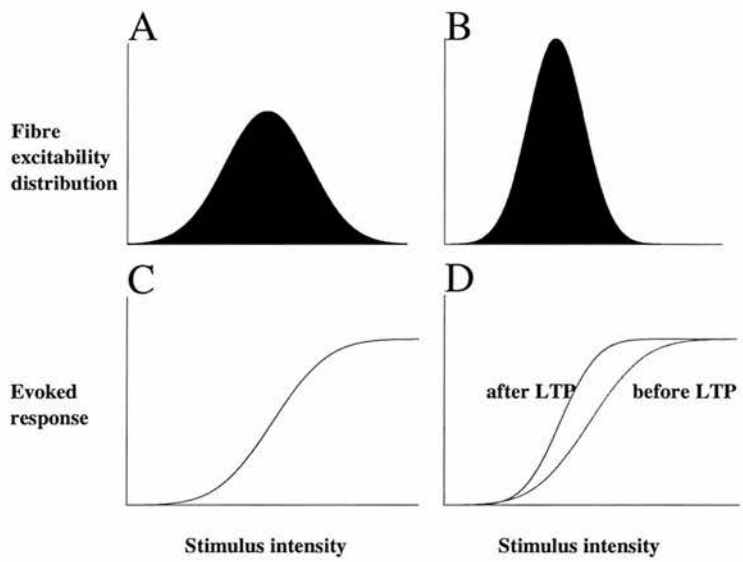


Figure 5.30 Effect on the shape of the IO curve of the distribution of firing thresholds of afferent fibres in a bounded region of tissue. The ordinate represents current intensity of increasing strength and the abscissa represents the number of fibres possessing a given threshold (A and B) or crossing the threshold to produce axonal action potentials (C and D). The upper graphs represent the distribution of firing thresholds while the lower graphs represent the number of fibres firing at that intensity: *i.e.* the cumulative sum of the distributions above. The rightmost line in D represents the baseline value depicted in C. A broad distribution of firing thresholds (A) results in a flat curve (C) while a reduction in mean firing threshold shifts the curve to the left and a narrowing of the distribution causes it to become steeper (B and leftmost curve in D).

In reality, the relative homogeneity of the perforant path fibre population, which has a low variance of fibre diameters (McNaughton *et al.*, 1981) means that the influence of the distribution of fibre excitability on IO curve shape is likely to be small, unless exceedingly low stimulus intensities are used. Furthermore, a post-tetanisation change in fibre excitability characteristics would be expected to change the size of the fibre

potential preceding the EPSP onset. Such a change is not observed following LTP induction (Andersen *et al.*, 1980).

Current spread and the limited size of the perforant path

The above explanation presumed a constant intensity of current across all fibres. However, there are two additional factors which also influence the shape of the curve. The first is that as stimulus strength is increased, fibres are recruited from an ever-widening cross-sectional area of the perforant path. Each new region is further from the electrode and the proportion of fibres which cross their firing thresholds will fall off rapidly as current density decreases with the square of the distance from the electrode.

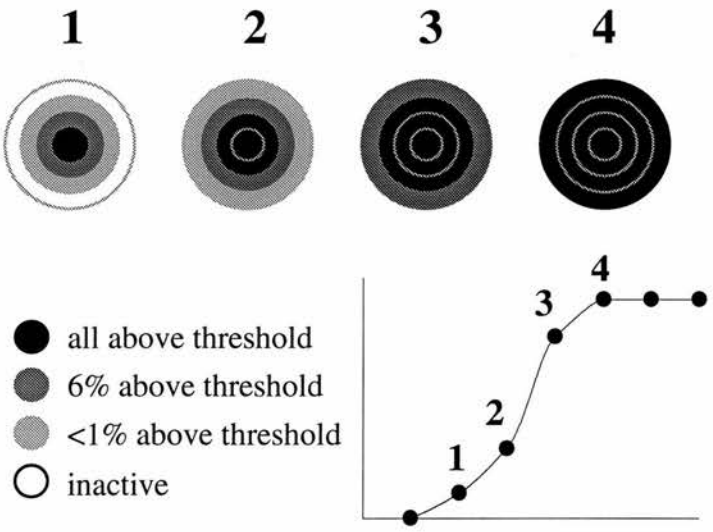


Figure 5.31 Contribution of current spread and the perforant path boundary to the shape of the IO curve. The concentric rings in the top row represent the perforant path in cross-section, with each shaded area representing a constant degree of activation (stimulus intensity increases from left to right). The corresponding positions on the IO curve of the resulting evoked responses are indicated by the same numbers as the circles. The percentage of fibres reaching firing threshold, as indicated by the legend, was calculated by assuming a Gaussian distribution as in the previous figure, with an inverse-square fall-off of current density with distance from the stimulating electrode. 1: a low current intensity stimulates only those fibres closest to the electrode tip. 2: increasing the stimulus strength results in greater current spread. The area of the concentric rings increases geometrically. 3: when current is sufficiently strong to spread to the edge of the perforant path, any further increases can only occur by increasing the proportion of fibres in the outermost region which cross firing threshold. Thus, the curve starts to flatten out. 4: when all fibres in the perforant path have reached threshold the curve becomes flat.

Counteracting this is the fact that the stimulated area increases geometrically with constant increases in current strength, and the summation over the Gaussian (see previous figure) is very steep as current intensity increases towards the mean firing threshold. Thus, as each region of the perforant path sees an increasing local current density with increasing stimulus strength, the number of fibres reaching threshold will escalate rapidly. The second factor is that the perforant path is not infinitely large but is bounded, so that a point is reached where no additional area is being involved by increasing the current strength. When this region is reached, further increases in evoked potential size can only

take place by increasing the proportion of fibres within the outermost region which reach threshold, thus flattening the IO curve. When this value reaches 100% then no further increases can take place.

Synaptic strength

A further factor governing the size of the evoked response following a given stimulus pulse is the strength of the synapse between the fibre terminals and the postsynaptic cell. The term "synaptic strength" refers to a collection of factors including number and size of synaptic vesicles, probability of transmitter release and postsynaptic sensitivity, the combined effect of which is to multiply the fibre potentials by some amount. Long-term potentiation involves a change in synaptic strength following tetanisation. In the present experiment it was found that the post-tetanisation increase in the size of the evoked response declined with increasing stimulus intensity. One possible explanation is that more of the synaptic terminals of fibres close to the stimulating electrode were potentiated than of those further away, so that low-intensity test pulses recruited a higher proportion of potentiated fibres than high intensity pulses (Fig. 5.32)

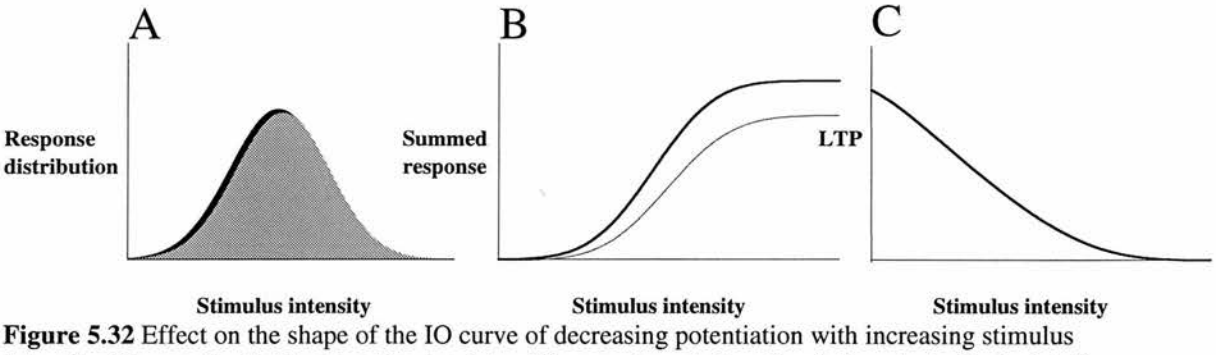


Figure 5.32 Effect on the shape of the IO curve of decreasing potentiation with increasing stimulus intensity. Fibres activated by low stimulus intensities are closer to the stimulating electrode. In this figure the amount of potentiation was highest for these fibres and steadily reduced for those more distant (A), resulting in a convergence of the pre- and post-tetanisation IO curves (B). The amount of resulting potentiation is shown in C.

Such a distribution of potentiation might occur if the stimuli during the tetanus had been insufficiently strong to activate 100% of those fibres which were stimulated by the strongest pulses on the IO curve, thus allowing the synapses of some of the more peripheral fibres to escape potentiation. It is not known whether the tetanus intensities used in the present experiment were producing maximal responses (though it is likely). It could be argued that if the tetanic stimuli were failing to produce near-maximal responses as measured on the IO curve (something which was not determined in this experiment), then they would have produced only incomplete perforant path activation, and the decline of potentiation with increasing current strength during IO curve measurement might therefore be due to recruitment of some of these unpotentiated synapses. However, several experiments in which the tetanus pulse intensity *was* established to be well above

that needed to evoke a maximal response have also found a decline of potentiation with increasing current strength (e.g. Cain *et al.*, 1993, Robinson, 1992). Furthermore, because fibre action potentials occur with a threshold stimulus intensity, above which potentiation in their synaptic terminals will occur (assuming uniform and sufficient postsynaptic depolarisation) and below which it will not, it would be expected that if all the fibres close to the stimulating electrode were potentiated while some further away were not then the decline of LTP should be a step function. In other words, 100% potentiation should be measured until the place on the IO curve at which some of the fibres started dropping below their activation thresholds, at which point measured potentiation should suddenly start to decline. Recall from Fig. 5.10 that the decline in potentiation started from the very beginning of the IO curve and was smoothly exponential, with no suggestion of a discontinuity. It therefore must be the case that if residual unpotentiated synapses are causing the decline in LTP, the failure to potentiate must involve even fibres very close to the electrode tip, which are receiving the highest current intensities. If even some of *these* fibres are failing to potentiate then it is doubtful whether any stimulus intensity could saturate LTP in this pathway. In other words, merely setting the tetanus pulse intensity to evoke a "maximal" response as measured on the IO curve would not necessarily guarantee complete potentiation of all synapses, assuming the above explanation and given the pattern of LTP induction seen in this experiment.

Be that as it may, the existence of a steadily decreasing proportion of potentiated synapses with more distant fibre recruitment might explain the decline in LTP across the IO curve. A more puzzling problem, though, is why the rate of this decrease would correlate with initial magnitude. In other words, why should it be the case that the more potentiated the local fibres, the *less* potentiated the more distant ones? The correspondence of rate of decline with magnitude suggests that a single process underlies both phenomena. For this reason, it seems that the most plausible explanation of the decline of measured LTP with increasing stimulus intensity involves the final determinant of IO curve shape, the non-linear summation of postsynaptic potentials.

Non-linear summation of postsynaptic potentials

Non-linear summation refers to the interference of convergent PSPs with each other and was briefly discussed in Chapter 1. The typical effect is to reduce the size of the composite evoked response as compared to the algebraic sum of the unitary potentials when each is evoked independently, though under certain circumstances the response may be larger than would be predicted. Redman (1976) discussed two main reasons for the effect: first, the potential produced by each synaptic event may shift the postsynaptic cell further towards or away from reversal potential and hence lower the driving force for the ion influx produced by the other(s). This means that postsynaptic potentials can mutually

interfere even if they synapse on different dendritic arbours of the same cell. Second, the ion channel opening produced by each potential increases the local membrane conductance and hence lowers the voltage change produced by the ion influx. This is a phenomenon known as shunting, and accounts for the fact that some types of inhibitory input may counteract simultaneously-occurring excitation even when the cell is near the reversal potential for the inhibitory ion current and hence experiences little or no inhibitory hyperpolarisation, or even a *de*polarisation (if the cell was on the far side of the inhibitory reversal potential). Shunting occurs independently of whether the potentials are inhibitory or excitatory, but only when they are evoked on neighbouring regions of the dendritic membrane. If there is a great deal of convergence of the axon terminals onto granule cells then the damping effect of both types of non-linear summation will be greater.

McNaughton and Barnes (1977) observed non-linear summation of EPSPs when two spatially separate perforant path stimulating locations were used, and suggested that this phenomenon be used as a criterion for pathway convergence. McNaughton *et al.* (1981) investigated EPSP summation in the dentate gyrus in hippocampal slices, and concluded that non-linearity was greater for quanta released at a single synaptic site than for those released at spatially separate sites. Langmoen and Andersen (1983) investigated summation of EPSPs evoked in separate parts of the dendritic tree of pyramidal cells and found that while small-amplitude EPSPs summated linearly, larger EPSPs summated non-linearly except in the presence of picrotoxin. It therefore appears that the addition of IPSPs may account for the observed non-linearity for spatially separate EPSPs.

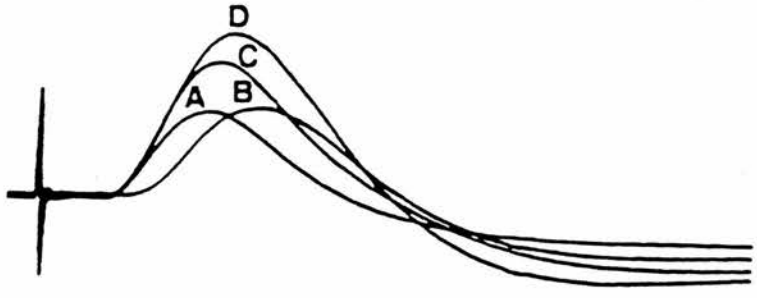


Figure 5.33 Non-linear summation of EPSPs (taken from McNaughton and Barnes, 1977). The waveforms marked A and B were evoked by different stimulation sites in the perforant path. C represents the response obtained when both pathways were fired simultaneously and D is the computed sum of A and B.

Non-linear summation could contribute to the flattening of the IO curve because increasing recruitment of perforant path fibres increases the number of converging inputs which activate dentate granule cells. After tetanisation, when excitatory and possibly inhibitory PSPs have been strengthened, it may be the case that non-linear summation

could be sufficiently large as to start to override, at the highest stimulus intensities, the increase in evoked potential size produced by the potentiated synapses. In other words, under the artificial conditions produced by massive synchronised activation of perforant path afferents by strong electrical stimulation, the effect of LTP is to undermine itself at high intensities so that at the top of the IO curve there is little or no measured potentiation. Such a situation would explain why the magnitude of potentiation and rate of its decline correlate so highly: the greater the potentiation, the greater the associated non-linearity and hence the more the PSPs interfere with each other as current strength increases.

One difficulty with this argument is that the non-linear summation observed in the experiments described above was only ever observed near or after the peak of the EPSP, whereas EPSP slope in the current study was always measured early on the field potential near the onset of the rising phase (see Fig. 2.1), where the contribution of IPSPs is minimal or absent. It is somewhat difficult to explain why early non-linear summation should occur in the present experiments even though it has not been observed previously. One possibility is that the effect does not become observable on the early phase of the EPSP until the current strength becomes sufficiently intense. In none of the above studies were current strengths used that were sufficiently intense to evoke a population spike. As stimulation intensity increases and a population spike starts to appear, it may be that early firing granule cells (Andersen *et al.*, 1971a) produce an occult flattening of the EPSP slope which behaves in a non-linear manner. In addition, possibly current strengths of this magnitude are required in order to produce sufficient convergent activation to allow EPSP shunting or feedforward inhibition.

If non-linear summation explains the decline of LTP across the IO curve, then the most accurate measure of LTP would be the magnitude measured at very low stimulus intensities, where the number of activated fibres is small and hence the convergence low. It is difficult to make measurements at these low intensities because field potential recordings in awake animals are variable and easily obscured by noise. However, the knowledge that there is a systematic relationship between LTP and current strength at higher intensities means that an estimate of what the LTP level *would* be at low intensities can be obtained by backwards-extrapolating the LTP/current regression line to the *y*-intercept (Fig. 5.19). This value represents the theoretical LTP that would be measured at 1 μA, or perhaps following activation of a single fibre. If this is accepted as a better estimate than the contaminated measure obtained with stronger test pulses then it appears that EPSP LTP in awake rats is typically of the order of 100-300%: that is, more commensurate with the estimates obtained from *in vitro* recording (*e.g.* Barrionuevo and Brown, 1983). Note that the spike potentiation is considerably higher, of the order of 10^{10} at the *y*-intercept. This is possibly because the spike, being a thresholded event, starts

from a baseline exceedingly close or equal to zero (*i.e.* non-firing of the postsynaptic cell). The faster decline of the spike may have its origins in the extra shunting and/or hyperpolarisation conferred by the longer-latency IPSPs.

5.4.2 Correlation of LTP with learning

Correlation of LTP with spatial learning

The second major finding of Experiment 4 was that LTP after 5 days of tetanisation correlated significantly with spatial learning ability. However, the correlation contained a surprising contradiction. When LTP was measured using strong test pulses (1000 μA), the relationship was positive: that is, the best-learning rats showed the greatest level of LTP. This replicates the findings of Experiments 1 and 2 and is in accordance with computational models of learning, which would predict that since synaptic plasticity putatively mediates learning, then the rats with the most plastic synapses should show both the greatest LTP and the best spatial performance. However, when LTP was measured using low strength test pulses (200 μA) the correlation reversed, and the rats which had been showing the greatest LTP with the stronger current showed the least with the weaker current. This occurred because of the relationship discussed in the previous section, between the rate of decline of LTP across the IO curve and its magnitude at low intensities. When LTP was adjusted as described in the previous section for its variation across the IO curve, the two parameters describing its behaviour, the slope and *y*-intercept of the linear regression line, each correlated with spatial learning.

The IO curves of both EPSP and population spike for good and poor learners crossed at a stimulus intensity of approximately 400 μA, which is the middle region of the curve where LTP is typically measured. This fact may explain why the correlations in the present study became weaker with successive experiments: current intensities were progressively reduced as implantation improved with practice and more robust population spikes were obtained, thus probably shifting the region of measurement from the high region of the IO curve towards the crossover point. It may also explain why a correlation such as this has not been reported by others. Only two previous studies appear explicitly to have correlated LTP with learning across a range of stimulus intensities, those of Robinson (1992) and Cain *et al.* (1993). In the Robinson (1992) experiment, the number of trials to criterion of rats performing a radial arm maze task was correlated with LTP of both EPSP and population spike across 5 stimulus intensities. LTP of the EPSP generally correlated positively with the number of trials to criterion (in other words, negatively with spatial learning ability) though the correlation was not significant. LTP of the population spike, by contrast, showed the reverse relationship. There was no indication of a crossover of the curves such as that found here. One possible explanation is that the 5

stimulation intensities used were all situated below the crossover point for the EPSP, but some were past the crossover for the population spike. Examination of Figure 1 of that study (p. 391) suggests that none of the stimulation intensities used evoked a maximal EPSP but the highest intensity may have evoked a maximal population spike (because spike IO curves plateau sooner than EPSP curves). However, because the spatial task used was different from the present one and only one of the correlations performed reached statistical significance it cannot be determined whether the findings of that study reflect, contradict or are neutral with respect to the present findings.

The other study, of Cain *et al.* (1993), correlated LTP measured at 3 different stimulus intensities with either prior or subsequent watermaze performance. Again, no more of the correlations were significant than would be predicted by chance, namely 3 out of 240. However, all 3 significant correlations concerned LTP induced after training, as in the present experiment, and of these, two were positive and one negative. Although the authors did not elaborate, it may be speculated that the negative correlations concerned the lower stimulus intensities and the positive correlation the highest intensity. However, at present it must be concluded that the findings of the present experiment are not supported by those of previous studies.

It was argued in the preceding section that a preferable measure of LTP would be one which was independent of the variation of potentiation across the IO curve. One such measure is the *y*-intercept of the linearised LTP/current relationship. Using this measure, it would appear that LTP is enhanced in rats which are poor learners relative to those which are good learners. Possible reasons for this apparently paradoxical finding are addressed in the next chapter.

Correlation of LTP with non-spatial learning

After spatial training and tetanisation had been concluded, the rats were trained on a simple conditioning task to see (a) whether ability on the spatial task predicted ability on this task and (b) whether LTP correlated with the latter. Given that LTP correlated with spatial learning ability, it follows that if (a) was true then (b) also would be true. However, although a similar pattern of IO curve convergence was seen between good and poor learners (Figs. 5.23 and 5.24), neither spatial learning ability nor LTP induction correlated significantly with discrimination performance (Figs. 5.5 and 5.26). Of the good spatial learners, half were poor discrimination learners and half were good. Likewise, of the good Skinner box learners, half were poor spatial learners and half were good. Three rats were poor at both tasks. One possible explanation for these findings was that a potential correlation with spatial learning was obscured by a floor effect: in other words, conditioning did not proceed for a sufficient length of time to separate the true good learners from the true poor learners. Arguing against this is the fact that the good Skinner

box learners were significantly better than the poor learners, suggesting that sufficient learning had taken place in at least some animals.

Although there was no correlation between performance on the spatial task and performance on the conditioning task, nevertheless the IO curves of good and poor Skinner box learners showed pattern similar to that of the good and poor spatial learners: that is, a faster convergence in the poor-learning animals than in the good learners. This suggests that a major contribution to the IO convergence pattern came from animals which were in the good and poor learning groups for both tasks. In order to test this possibility, the LTP analysis was repeated just for those animals which were good or poor at both tasks, whereupon the LTP/learning correlation for these animals increased markedly. The tentative conclusion, then, is that LTP induction correlates best with some learning competence which is common to both the spatial and the non-spatial tasks.

Effect of LTP induction on simple conditioning

Induction of perforant path LTP did not appear to affect discrimination learning in these animals, unlike the rabbits in the Berger (1984) eyeblink conditioning study. There are several possible reasons for this. First, the function of hippocampal synaptic plasticity in rats may differ from that in rabbits, though this is arguably an unlikely cause given other similarities of hippocampal physiology between the two species. Second, the eyeblink conditioning task may require an anatomically different substrate for its representation than the tone-click discrimination used here, even though the two tasks are generally considered to be representative examples of classical conditioning. The fact that one task is aversive and one appetitive may mean that they are processed differently in the brain. Eyeblink conditioning is also considerably slower, requiring hundreds of trials as compared with the 60 trials used in the tone-click discrimination, and so is perhaps more sensitive to the disruptive effects of hippocampal stimulation. The Berger study has not been replicated and remains a curiously anomalous finding in the LTP-behaviour literature. Given that it now represents the only convincingly documented example of LTP-induced behaviour change (other than the tetanisation-as-CS studies discussed in Chapter 1), it merits further investigation.

5.4.3 Low-frequency spike potentiation

The final and unexpected finding of Experiment 4 is of a very significant enhancement of the population spike following a stimulation protocol which had been intended as a control condition (Fig. 5.6). The low frequency stimulation consisted of bursts of 5 strong 1 Hz pulses given at 1 min intervals. Although the elevated post-stimulation values were recorded 30 min after the end of the stimulation session, suggesting a process with a time course comparable to that of LTP, the EPSP was not affected and so the phenomenon

does not appear to be an LTP-like potentiation. It may, however, represent the E-S component of LTP, which usually occurs with a higher threshold than LTP (Abraham, 1984, Abraham and Goddard, 1984) but is sometimes observed in the absence of EPSP potentiation (Bliss and Lømo, 1973).

Stimulation of the perforant path at 1 Hz is known to produce changes in the subsequent evoked response which include depression of both EPSP and population spike (habituation), accompanied by an enhancement of the population spike relative to the EPSP (E-S potentiation, Abraham and Bliss, 1985). Prolonged stimulation may also induce a long-lasting homosynaptic depression via an NMDA-receptor-dependent mechanism (Dudek and Bear, 1992). It may be that inhibition habituates at a lower threshold than excitation, producing population spike enhancement in the absence of any other observable changes. Only one stimulation pathway was used throughout these experiments and so there is no way to determine from present data whether the change was homosynaptic or heterosynaptic. It may be due to a generalised increase in cell excitability following the application of strong pulses to the perforant path. The striking feature of the phenomenon, however, was that despite the application of the same stimulation protocol on each of 8 successive days, the increase became progressively less over subsequent days and was virtually nil by the last day.

Because the stimulation was applied on the first day after watermaze training, a possible explanation both for the increase and for its subsequent decline is that spatial training somehow interacted with low-frequency stimulation occurring immediately afterwards but not after several days. Thus, as the length of the interval between training and stimulation increased the interaction and consequent spike potentiation faded. In order to test this possibility an additional 3 animals were included in the low-frequency group. These animals had had both spatial training and LTP induction in a previous experiment, but their potentials had decayed back to baseline. The same increase was seen following low frequency stimulation. Thus if prior training had been interacting with low frequency stimulation in order to cause the potentiation, its effects would have to have lasted for many weeks, a somewhat unlikely possibility. A more plausible explanation is that some change in postsynaptic receptor sensitivity occurred with successive stimulations so that the potentiating effect of the low frequency trains became less efficacious with time.

This finding offers a potential explanation for the very abrupt saturation of spike potentiation seen under burst-train conditions used here and in Experiments 1 and 3. The burst-train condition was identical to the low frequency protocol used here except that each single pulse was replaced by a 400 Hz train. Suppose this protocol is capable of inducing the same pattern of decrescendo spike potentiation as the low-frequency condition, via a long-lasting modulation of transmitter systems or some related

mechanism. This effect would be masked by concomitant LTP resulting from the high frequency component of the trains, but might manifest itself in certain ways. For example, the acquisition of population spike LTP might be initially fast but slow down as the decline in "low-frequency" potentiation took hold. The result might be to produce an initial sharp rise in population spike potentiation followed by an early plateau and perhaps even subsequent decline. Exactly this pattern was seen in Experiments 1 and 3 (Figs. 3.2 and 4.3), contrasting with the much more gradual plateau seen in Experiment 2 (spaced protocol), where the spike continued to increase over several more days (Fig 3.6).

The finding that low frequency stimulation is capable of producing such long-lasting changes in hippocampal physiology is of some importance in studies of hippocampal plasticity, since these changes do not necessarily involve the NMDA-associated LTP-generating system and yet may interfere with the recording of "baseline" responses. The finding that low frequency perforant path activity could alter transmitter systems would add an extra dimension to studies of perforant path plasticity, and for this reason the phenomenon may deserve further investigation.

5.5 Contribution of NMDA receptors to LTP and learning

The results of Experiment 4 supported the findings of Experiments 1 and 2 of a relationship between LTP magnitude and spatial learning. One question which arises from this finding is the physiological nature of the connection: that is, what determines the magnitude of LTP after repeated tetanisation, and is this factor also related to learning? One obvious possibility is that both spatial learning and LTP magnitude are dependent upon NMDA receptor function. An attempt was therefore made to assess the contribution of NMDA receptor current to the evoked potential during a tetanus in good and poor spatial learners, to see if this parameter could explain the LTP/learning correlation seen in the previous experiments.

The animals used in this experiment were 15 subjects from Experiment 4. Nine of these had been low-frequency (LF) control animals and 6 had been HF animals whose evoked responses had returned to baseline. The purpose of the experiment was to use a method described by Abraham and Mason (1988) to obtain an evoked response during a tetanus both with and without a contribution from NMDA receptors, in order to compare the responses and ascertain what proportion of the response during a normal tetanus was composed of NMDA current. Because the spatial learning ability of these animals had previously been determined, a comparison could then be made of the relative NMDA contribution between animals of good and poor spatial learning ability.

NMDA receptors were blocked by means of *i.p.* administration of the competitive antagonist 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (CPP-ene). This

agent is a potent anaesthetic and interacts with standard anaesthesia to produce profound respiratory depression. A preliminary trial using three animals from Experiment 2 suggested that the dose of CPP-ene required to block NMDA receptors reliably was lethal unless very light anaesthesia was used. Therefore, the animals were anaesthetised to no more than a state of light unconsciousness. Because no surgery was involved this was sufficient to stabilise the evoked responses.

5.6 Experiment 5 Methods

Because of their participation in Experiment 4, the animals had already been implanted with chronic electrodes in the perforant path and dentate gyrus as previously described. Each animal was removed from its home cage, weighed and lightly anaesthetised with urethane, so that it was lying quietly and breathing evenly but responsive to a tail pinch. Atropine was also administered to minimise respiratory secretions. The animal was wrapped in cotton wool and a rectal thermometer was inserted. Core body temperature was monitored very carefully throughout the course of the experiment and kept to within 0.3°C of its baseline value (around 36.5°C) by means of a heating lamp in order to assure stability of the baseline evoked responses (Moser *et al.*, 1993). The headcap was connected to the standard recording apparatus and the stimulus intensity adjusted to give a population spike of 1-3 mV in the usual manner.

The time course of electrophysiology and drug administration was as follows. A 30 min baseline was collected, consisting of single pulses evoked once every 40 s. If the response showed signs of instability or if the temperature fluctuated more than 0.1 or 0.2 degrees the baseline was restarted. When a stable baseline had been collected a set of 5 trains was applied at 1 Hz. A train consisted of 10 pulses at 400 Hz, as in the previous experiments. A post-tetanus baseline was collected for a further 30 min and then CPP-ene administered *i.p.* at a dose of 10 mg/kg. Further trains were then given as before at 60 and 90 min post-drug injection. The animal was then killed with an injection of sodium pentobarbital and perfused.

5.7 Experiment 5 Results

Despite the anaesthetic precautions taken, 3 animals died at around 60 min post-drug injection, two from the ex-LF and 1 from the ex-HF group. The data from a third ex-LF animal were corrupted on disk. There are therefore data from 6 ex-LF and 5 ex-HF animals. Analysis of the data after the experiment was completed showed that two rats, one from each group, had unacceptably unstable baselines. These animals have been excluded from the group analysis but their data points have been indicated on the correlogram below.

5.7.1 Effect of CPP-ene on evoked responses and LTP induction

The first set of trains evoked robust LTP of both the EPSP and population spike (Fig. 5.34), as measured by the average of the last 10 pulses prior to drug injection.

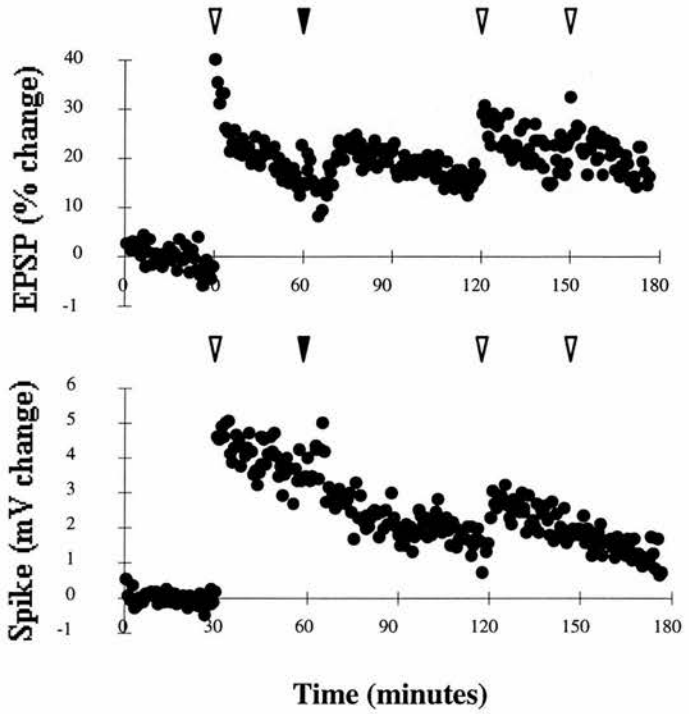


Figure 5.34 Effect of an *i.p.* injection of CPP-ene on subsequent evoked responses and LTP induction. The hollow arrows represent tetanisation episodes and the filled arrow the time of drug administration. Robust LTP of both EPSP (A) and population spike (B) followed the first set of trains 30 min after the onset of baseline recording. Drug injection 30 min after the first tetanus was associated with a transient decline in the EPSP and a prolonged decrease in spike amplitude. A second tetanus at 60 min post-injection (time = 120 min) produced a slight amount of LTP whereas a tetanus 30 min later (time = 150 min) produced no further change.

Values for EPSP and population spike potentiation were 16.22 (±3.19) % and 2.94 (±2.13) mV respectively. Paired *t*-tests confirmed that the increase was significant for both parameters ($t = 5.66$, $p < 0.0001$ for the EPSP; $t = 7.74$, $p < 0.0001$ for the population spike). Administration of the drug produced a transient fall in the size of the EPSP and a prolonged fall in the population spike (see also Abraham and Mason, 1988). The first post-drug set of trains, at 60 min after injection, produced a significant further increase in the EPSP ($t = 2.59$, $p < 0.05$) and population spike ($t = 2.63$, $p < 0.05$). A further set of trains at 90 min produced no further change in the size of the EPSP when measured 30 min later ($t = 0.73$). The population spike was significantly smaller at this time ($t = -3.07$, $p < 0.05$), reflecting the drug-induced downwards drift.

5.7.2 Effects of CPP-ene on intra-tetanic waveforms

The effects of CPP-ene on the shape of the intra-tetanic waveform are illustrated by the two examples in Fig. 5.35. Ninety min after drug injection the response evoked by the

high-frequency trains is considerably smaller. The difference from the pre-drug response is illustrated by the lower waveform, and is largely due to the contribution of NMDA receptors to the pre-drug but not the post-drug response during a tetanus.

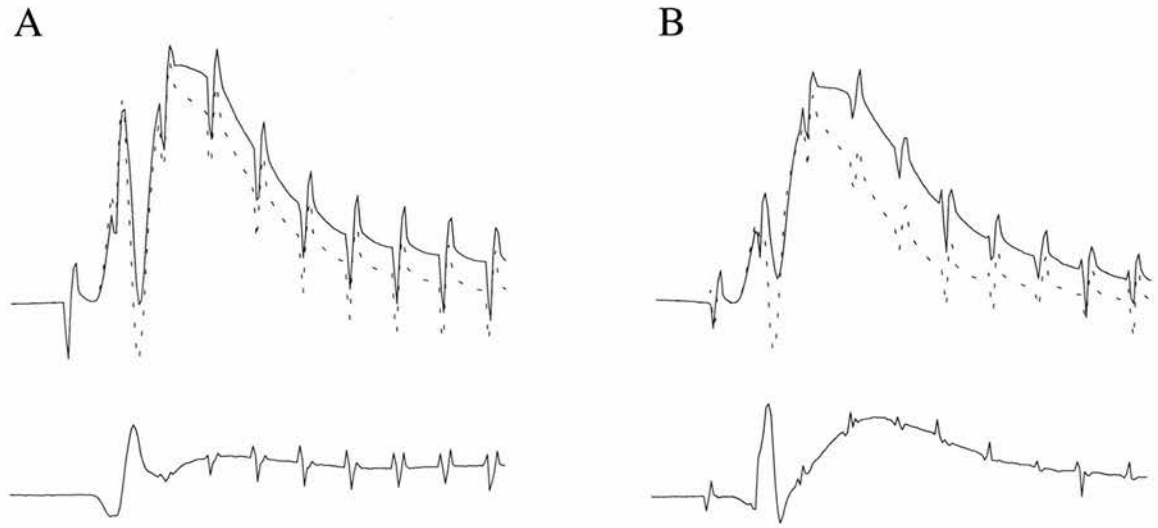


Figure 5.35 Top traces: examples of evoked responses during a tetanus before (solid line) and after (dotted line) NMDA receptor blockade with CPP-ene. The lower traces were obtained by subtracting the post-drug from the pre-drug traces. (A) the waveforms from an animal with a small NMDA receptor component to the tetanic evoked response. (B) waveforms from an animal with a large NMDA component.

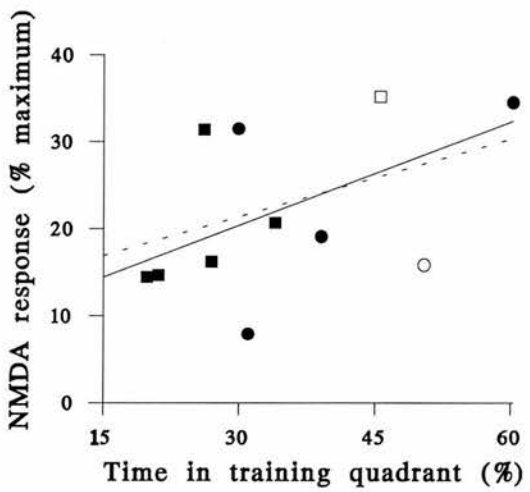


Figure 5.36 Correlation between the maximum NMDA potential and previously-determined spatial learning ability. Squares = ex-LF animals, circles = ex-HF animals. The two hollow symbols represent animals with unstable baselines. The solid line is the regression line without and the dotted line the regression line with these two animals. Although the correlation was positive it did not reach statistical significance.

5.7.3 Correlation of NMDA response with spatial learning

The size of the NMDA response was quantified by determining the maximum height of the NMDA potential of the first train of the 90 min post-drug tetanus, and expressing it as

a percentage of the maximum height of the response during the first train of the pre-drug tetanus. The individual values are plotted against spatial learning ability of the rat, as shown in Fig. 5.36. The correlation was positive but not significant ($r = 0.52$, NS).

5.8 Discussion of Experiment 5

The aim of Experiment 5 was to determine whether the greater levels of LTP seen in the poor learning rats in Experiment 4 were due to an obvious superiority, in these animals, of the NMDA current evoked during a tetanus. In fact, quite the opposite was found: poor learning animals possessed *smaller* NMDA currents than the good learners. It thus appears that the enhanced LTP of the poor learners cannot be attributed to greater NMDA function.

The finding of a positive correlation between spatial learning and NMDA current size must be treated with caution, since the relationship did not reach statistical significance. It does, however, raise the intriguing possibility that the enhanced LTP seen in the poor learners may have arisen as a compensatory mechanism to counteract the effects in these animals of their smaller intra-tetanic NMDA currents. Recall that the LTP in Experiments 1 to 4 of this study had been measured after several days of induction, and so represents a (possibly local) maximum or "ceiling" value. The factors controlling this ceiling level are currently unknown, but one possibility is that the level is tied to LTP induction, and that variations in one parameter are compensated by inverse variations in the other in order to keep synaptic efficacy changes within an optimal range. In other words, rats in which LTP is hard to induce because of small NMDA currents may show a compensatory increase in LTP expression, which becomes revealed as enhanced potentiation after several days of tetanisation.

It was suggested in Chapter 1 that although the term "synaptic plasticity" is commonly used interchangeably with "LTP" to refer to synaptic efficacy changes, a distinction should be made between these terms and "plasticity" should be used to refer to the readiness of a synapse to change strength, independently of any actual efficacy changes. The reasons for making this distinction are first, that a synapse may be plastic without necessarily having changed its strength, and second, that LTP induction probably has a different mechanism from expression. The results of Experiments 4 and 5 support the latter hypothesis. The focus of molecular biological LTP experiments is currently turning towards mechanisms of expression, in which the distinction between plasticity and LTP expression becomes of great importance. The possibility that these two factors are inversely modulated merits further attention.

Chapter 6 – General Discussion

6.1 Summary of findings

The aim of this thesis was to explore the possible relationship between experimentally-induced changes in synaptic strength and learning. There are two main results:

(1) experimental induction of synaptic efficacy changes to an apparent maximal level did not affect spatial learning, and

(2) the amount of synaptic efficacy change induced in a given animal correlated with its spatial learning ability.

The issue of whether changes in synaptic strength mediate learning is important, because either a positive or negative answer would greatly constrain the types of theoretical model which could plausibly explain hippocampal function. The question now concerns the status of the synaptic plasticity/learning hypothesis, when the results presented here are taken in conjunction with other recent findings regarding the relationship between hippocampal evoked potentials and learning.

The present study examined the plasticity/learning hypothesis in two different ways. First, an attempt was made to replicate the disrupting effect of LTP on spatial learning reported by McNaughton *et al.* (1986) and Castro *et al.* (1989). Second, a correlation between LTP magnitude and learning ability was discovered and explored. The implications of the findings of these two approaches for the hypothesis are discussed below.

6.2 LTP saturation

At the time that this study began, some of the most convincing evidence in support of the plasticity/learning hypothesis came from the occlusion studies of McNaughton *et al.* (1986) and Castro *et al.* (1989), where it was found that tetanisation of the main input pathway into the hippocampus produced a substantial impairment of subsequent spatial learning. The strength of this finding lies in the proximity of the intervention (*i.e.* the tetanus) to the postulated site of its action (the perforant-path/dentate synapse), as contrasted with other types of less specific manipulation such as drug injection. Because of this proximity, and the assumed specificity of the effect, there are relatively few alternative explanations for the learning impairment. Tetanisation is a comparatively easy treatment to administer, and so the occlusion effect appeared to offer not only strong

support for the hypothesis but also a useful tool with which to explore further the subcomponents of spatial learning.

The first two experiments reported here attempted to replicate the effect using a watermaze task similar to that used by Castro *et al.* In the first experiment, a modified protocol was employed in order to avoid some of the potential problems of their methodology, such as the temporal proximity of tetanisation to behavioural training. Because no effect of tetanisation on learning was seen, a second attempt at replication was made using a protocol as nearly as possible identical to theirs. Again, no learning impairment was produced.

At the time that these experiments were taking place, several other groups were also trying and failing to replicate Castro *et al.* A synopsis of these studies is presented in the Appendix, and possible reasons for the widespread inability to produce a tetanisation-related spatial learning blockade were discussed in Chapter 3. Essentially, there are two possible explanations: either the findings of the original two experiments were spurious, or the findings were valid but dependent on some as-yet-unidentified aspect of the methodology of that laboratory, which was not replicated by any of the other groups. At present it is difficult to say which of these reasons is the more likely. Between them, McNaughton *et al.* and Castro *et al.* used three different spatial learning tasks and several different tetanisation protocols, with varying degrees of LTP "saturation" and tetanisation-training intervals. This fact, combined with the statistical power of the findings and their intuitive plausibility, led to a widespread belief in the validity of the results of both sets of experiments and a current reluctance to abandon the occlusion effect without further investigation. Some possible alternative strategies for future experimentation are discussed at the end of this chapter.

6.3 LTP/learning correlation

Although the first two experiments in this thesis did not substantiate the occlusion effect, they produced a finding which in itself supports the plasticity/learning hypothesis: namely, that the magnitude of accumulated LTP correlated highly with spatial learning ability. This finding is in agreement with other correlational findings such as between LTP duration and memory retention (Barnes, 1979, Barnes and McNaughton, 1985), or between the magnitude of weakly induced LTP and watermaze performance in young and old rats (Deupree *et al.,* 1991). In all of these experiments a positive correlation was found between LTP parameters and learning, supporting the hypothesis that synaptic modifiability should underlie learning ability.

Subsequent experiments in the present work, however, raised several questions concerning this relationship. Specifically, these later experiments suggested that the LTP

estimation may have been influenced by a measurement artefact, and that what had appeared to be a positive correlation was, in reality, a *negative* correlation. This paradoxical situation arose because of a correlation between LTP magnitude and the shape of the IO curve, so that rats with the greatest amount of LTP measured using small stimulus intensities showed the fastest convergence of pre-and post-tetanisation curves, and hence the smallest amount of LTP using large stimulus intensities. This unexpected finding raises questions both about LTP measurement and about the relationship between synaptic plasticity and learning. These two questions are addressed below.

6.3.1 Quantitative LTP estimation

The first question concerns the issue of how to make an accurate quantitative determination of LTP magnitude. The convergence of post-tetanisation IO curves towards the pre-tetanisation curves with increasing test pulse intensity has been informally well known for some time, but little discussed in the literature. Because the rate of convergence appears to vary between animals, measurement of LTP at a constant position on the IO curve (as is commonly done) would not be guaranteed to produce an accurate and reproducible LTP measurement, and therefore some other method of LTP determination is required. It was argued in Chapter 5 that it is preferable to use a parameter of LTP which produces a value consistent for a given animal, enabling valid between-animal comparisons of LTP induction. One such parameter is obtained by linearising the LTP/current curve, by means of log transformations, and taking as the measure of LTP a parameter of this line which is invariant, either its *y*-intercept (the theoretical potentiation which would be measured close to zero stimulus intensity) or the slope (rate at which measured LTP declines with increasing current).

It was further found that the rate constant of LTP decline is related to LTP magnitude, an observation which has not been reported previously and which suggests a common underlying factor. The hypothesis favoured by the discussion in Chapter 5 is that a population field potential is smaller than it "ought" to be because the unitary EPSPs which combine to make up the compound response are attenuated by the mutual interference contributed by shunting and possibly reduced driving force. The interference becomes greater with increasing current because more fibres are activated and hence convergence is higher, and it is related to LTP magnitude because large unitary EPSPs would be expected to interfere more than small ones. For this reason, a weak test stimulus should produce a more accurate estimate of LTP than a strong stimulus. Since the *y*-intercept represents the theoretical value of potentiation elicited by a near-zero stimulus intensity, where convergence is least, it would seem to be the most logical choice of LTP measure.

6.3.2 Relationship of synaptic plasticity to learning

One of the major findings of this thesis is that LTP magnitude correlated strongly with spatial learning ability. The results from Experiments 1 and 2 suggested that this correlation was positive: that is, that good learning rats showed greater LTP than poor-learning rats. The finding of a correlation between LTP induction and spatial learning has implications for the plasticity/learning hypothesis, which are discussed below. However, the results from Experiment 4 suggested that the correlation might in fact lie in the opposite direction to that which had initially been observed. If the argument is accepted that the *y*-intercept of the linearised LTP curve is the most appropriate measure of LTP induction, then it appears that synaptic plasticity is inversely correlated with learning in Experiment 4. On the basis of the findings of a change in IO curve shape after tetanisation, it therefore appears likely that the same relationship held in Experiments 1 and 2, where LTP was measured using moderately strong test pulses and a positive correlation was measured. The finding of an inverse LTP/learning correlation is contrary to what would be predicted by most computational theories of learning, and possible reasons for this apparently paradoxical finding are outlined at the end of this chapter.

6.3.3 Reasons for LTP/learning correlation

One reason why LTP magnitude correlates with spatial learning is that an LTP-like process subserves spatial learning under normal circumstances, and this is reflected in a parallel induction of artificially induced synaptic strength changes following tetanisation. This finding would be predicted by many computational theories of learning and therefore supports the idea that learning is indeed mediated by such synaptic changes. However, there are alternative explanations, some of which are as follows:

(1) LTP induction affects learning,

(2) Neurohumoral factors modulate both parameters together,

(3) Brain temperature affects LTP and correlates with learning, or

(4) There is a non-specific correlation of plasticity with learning.

LTP induction affects learning

One possible reason for the LTP/learning correlation seen in Experiments 1 and 2 was that the induction of LTP affected subsequent spatial learning by enhancing information flow through the dentate gyrus. This hypothesis was tested in Experiment 3, by reversing the order of training and tetanisation in some animals. A strong correlation failed to emerge in this experiment in either the rats trained first or the rats trained after tetanisation, and so no conclusions could be drawn about whether LTP could have affected learning in the first two experiments. However, with the more detailed

quantification of LTP employed in Experiment 4, an LTP/learning correlation reappeared even though the animals were trained prior to tetanisation. It thus appears that an LTP-associated learning facilitation could not explain the results of Experiments 1 and 2. In other words, the LTP/learning correlation would seem to be independent of the order of measurement of the two parameters.

The effect of LTP induction on a different kind of learning was explicitly examined in Experiment 4 using a classical conditioning task, which is a behavioural paradigm more closely related to that in which an LTP-related facilitation of learning has been previously reported (Berger, 1984). Rats in which LTP had been induced bilaterally to asymptote were trained on a simple tone-click discrimination. These rats performed no better or worse on the task than either low-frequency or unoperated controls, suggesting that LTP induction did not affect learning of this task. The implications of this finding for the Berger (1984) result are unclear, since a different species was used and the task was appetitive rather than aversive. However, it seems evident that even massive LTP induction has not affected learning in any of the rats in the present study.

Neurohumoral factors modulate both parameters together

A more plausible explanation for the LTP/learning correlation is that learning ability and synaptic plasticity are modulated by some non-specific regulatory influence which varies between animals such as arousal, alertness or stress. In Experiment 4, tetanisation was begun on the day after watermaze training and so LTP could either have been affected by the previous day's learning experience or by some feature of the general disposition of the rat which also affected how it performed on the spatial task. There is considerable evidence accumulating to suggest that LTP induction is affected by neuroendocrine factors related to an animal's reaction to stress and novelty. For example, rats exposed to an inescapable electrical footshock show impaired population spike LTP compared with controls exposed to the same number of shocks from which escape is possible (Shors *et al.,* 1989). Simple exposure to the electrophysiological recording environment appears to be sufficient to impair primed burst potentiation in the first few days of testing (Diamond *et al.,* 1990), and this effect may be modulated by adrenal stress hormones (Bennett *et al.,* 1991, Diamond *et al.,* 1989), such as opioids (Shors *et al.,* 1990).

With these findings in mind, it is possible that an experience such as the watermaze training procedure used here would constitute at best exposure to a novel environment, even allowing for pretraining, and at worst forced exposure to a series of aversive experiences (immersions) analogous to shocks. It is possible that depending on the learning ability of a given rat, the watermaze task resembles an escapable-shock task (for a good learner) or an inescapable-shock task (for a poor learner). One possible explanation for the correlation reported in Experiment 4 is therefore that the training

protocol used on the day preceding tetanisation stressed the rats by amounts which varied according to their learning ability and that this modulated subsequent LTP induction, producing different LTP in the good than the poor learners. To test this possibility, data were combined from Experiments 1, 3 and 4, in which the same watermaze training and LTP induction protocols were used, but with some rats trained before and some after tetanisation. Rats receiving training first showed slower acquisition of EPSP LTP [$F(5,175) = 3.26$, $p < 0.01$] with significantly different LTP levels by the second day of training (Fig. 6.1). However, levels had equalised between the two groups by the 5th day of tetanisation, which is the value used for the correlational analyses. Furthermore, spike LTP did not differ [$F(5,175) < 1$, NS], by contrast with the stress studies discussed above. It therefore seems unlikely that a stress-related LTP impairment could explain the distribution of LTP levels seen in the experiments in this study.

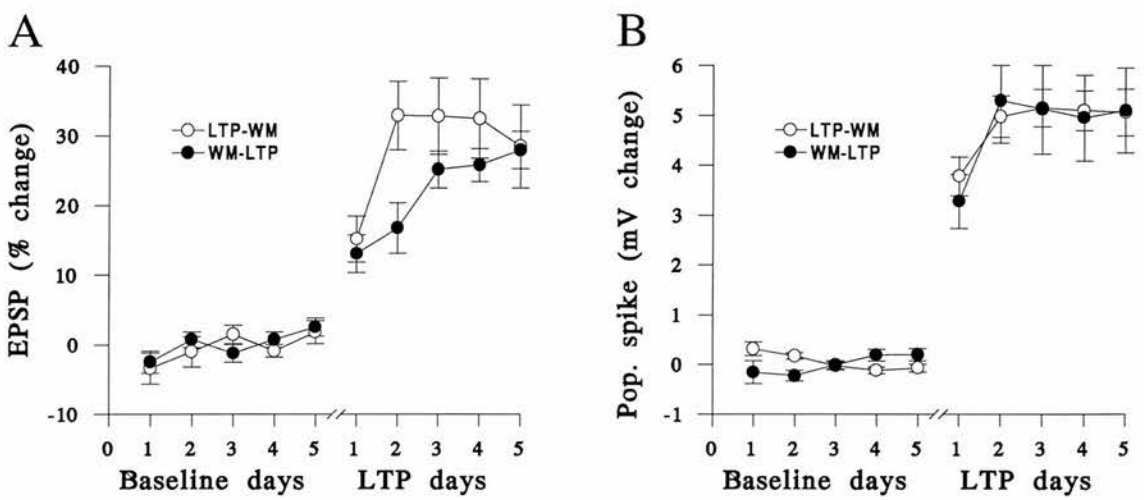


Figure 6.1 Comparison of LTP acquisition for EPSP (A) and population spike (B) for rats trained before or after LTP induction. Rats trained first (WM-LTP) showed slower acquisition of EPSP LTP than rats trained after tetanisation (LTP-WM). However, spike LTP acquisition did not differ.

Another argument against this hypothesis is the observation of a correlation in Experiments 1 and 2 when rats were trained *after* tetanisation. Under the reasonable assumption that the correlation seen in those experiments has the same explanation as in Experiment 4, it is therefore unlikely that the watermaze experience produced the observed distribution of LTP.

Alternatively, perhaps other aspects of the experimental protocol had a modulating effect on LTP, via a novelty-related mechanism similar to those in the primed burst experiments discussed above (Diamond *et al.,* 1989, 1990). It should be kept in mind that although watermaze training was the only time at which spatial learning was explicitly tested, nevertheless the experience of being transported to a new room and subjected to the electrophysiological recording procedures would constitute, for the rats, a significant learning experience (much of which, incidentally, is unavoidably spatial in nature).

However, in the Diamond *et al.* experiments primed burst plasticity was restored after sufficient acclimation to the recording environment, *i.e.* 7 h daily for approximately 7 days. Although recording sessions in the present experiment were much shorter, by the 5th day of tetanisation the rats had spent at least an hour in the recording chamber daily (except for the training day) for 10 days and seemed well habituated to the environment, often falling asleep if not watched carefully. Thus it seems unlikely that novelty-related stress could be the explanation for the LTP/learning correlation.

An alternative possibility is that the general disposition of the rat (*e.g.* whether it is naturally anxious or naturally phlegmatic) may contribute both to spatial learning performance and to LTP induction. Along these lines, a correlation was recently observed between exploratory behaviour and LTP induction that strikingly resembles that reported here (Maren *et al.,* 1993). Rats were rated according to their readiness to explore an unfamiliar alleyway, and two weeks later were anaesthetised and underwent perforant path LTP induction. The amount of time each rat had spent exploring the new environment correlated negatively with subsequent EPSP LTP and positively, in a separate group of animals, with levels of plasma corticosterone. The LTP showed both a lower induction threshold and a higher magnitude in the non-exploratory (neophobic) animals. Unfortunately, only the pre-tetanisation IO curves were reported and it is not known where the test pulses were situated on the curve. It is therefore not clear whether LTP estimation using the parameter established here would have correlated negatively or positively with neophobia. However, the test pulses were set to evoke a population spike of 2 mV which probably positions them somewhere in the lower region of the curve, assuming conditions comparable to those in the present Experiment 4. It could therefore tentatively be concluded that the neophobic rats in their experiment, showing better LTP and lower cortisol levels, correspond to the poor learners in the present study. One explanation for the present correlation is therefore that the disposition of the rats modulated both learning ability and LTP induction via neurohumoral factors related to affective responses intrinsic to the animals. Whether the learning effect *results* from the putative hormonal effect on synaptic plasticity or simply correlates with it is another question.

Brain temperature affects LTP and correlates with learning

A possibility closely related to the neurohumoral factors discussed above is suggested by the findings of Moser *et al.* (1993) that brain temperature correlates with both evoked potential size and physical activity. If rats with different learning abilities also differ in their natural activity levels, the result may be that LTP induction shows a secondary correlation with both of these parameters. Intracerebral thermistors were not used in the present experiments so it is not possible to assert with confidence that good and poor

learners did not differ in basal brain temperature. However, the temperature/physiology correlation observed by Moser *et al.* concerned baseline evoked responses, not LTP. Baseline evoked responses in the present study did not differ between good and poor learners, so if a temperature-associated difference existed between the two groups it would have to be so subtle as to be undetectable using ordinary physiological recording. In addition, it has not yet been determined whether temperature-induced changes in evoked response can modulate LTP induction. Alternatively, the onset of an activity-associated temperature difference may have occurred *after* spatial training, producing an increase in evoked response which resembled LTP. There are two reasons why this is unlikely. The first is that whereas temperature associated evoked response "potentiation" is accompanied by a decrease in population spike size, the population spikes in the current experiments showed an increase, suggesting that they demonstrated true LTP. Second, if the correlation in Experiment 4 has the same cause as that of Experiments 1 and 2, in which animals were spatially trained after tetanisation was completed, then a training-induced temperature difference could not explain it. It therefore seems unlikely that the present results are related to brain temperature differences between good and poor learners. However, until the experiment is replicated with concurrent temperature measurements the possibility cannot be entirely discounted.

There is a non-specific correlation of plasticity with learning

The finding that hippocampal LTP correlated with performance on a particular task in these experiments does not necessarily mean that the *hippocampus* mediates that task. The correlation found in these experiments could have arisen if general brain plasticity correlates with general learning ability. In other words, although the perforant path was chosen as the anatomical focus of study and spatial learning chosen as the task of interest and a correlation was found between the two, in fact any combination of pathway and task may have shown a similar correlation if plasticity and general learning ability ("intelligence") correlate throughout the brain in a given animal. This might occur if, for example, the determinant of plasticity (and hence learning) was some factor common to many or all plastic synapses, such as NMDA receptor density. If this is so then the present correlation does not provide specific support for the hypothesis that the hippocampus mediates spatial learning, though it does support the idea that plasticity of some form might do so. In order to determine whether hippocampal plasticity preferentially mediates spatial learning it is necessary to attempt to dissociate spatial from other forms of learning and show that hippocampal plasticity is related to the former and not the latter.

This reasoning was the motivation behind the inclusion of a non-spatial task in Experiment 4. The results of this part of the experiment are difficult to assess. There appeared to be no correlation between spatial and non-spatial ability, nor of non-spatial

ability with LTP. However, when the LTP/learning comparison was made for rats which performed well or poorly in *both* tasks the strength of the correlation increased considerably, even given the smaller numbers of animals involved. This suggests that there may be an occult interaction between the two tasks. For example, the watermaze task may possess elements of non-spatial learning (such as the formation of associations between multiple cues) so that animals which are naturally good at conditioning tasks will gain an advantage on the spatial task. Conversely, it is possible that optimal solution of the Skinner box task involves spatial (or spatio-temporal) reasoning. It may be that LTP (or rather, naturally occurring LTP) mediates either the non-spatial or the spatial elements of both tasks.

6.3.4 Reasons why the LTP/learning correlation is negative

The reasons why the LTP/learning correlation may be negative, in contradiction to theoretical predictions, are as follows:

(1) Learning occludes LTP induction,

(2) Hippocampal plasticity may mediate non-spatial learning,

(3) Plasticity upregulation is a compensatory mechanism,

(4) Absent-platform test does not measure only spatial learning, or

(5) Poor learners show impaired homosynaptic LTD.

Learning occludes LTP induction

Experiment 4 revealed that the LTP/learning correlation appears to be the inverse of that which would be predicted by theoretical models of learning. The first possible explanation for this observation is that the learning experience which took place prior to tetanisation occluded LTP by an amount proportional to the degree of learning which took place. This could happen if synaptic strength changes which occurred during learning reduced the range over which the same synapses could subsequently be shifted tetanically.

This argument is the converse of the saturation argument presented in Chapter 3, and can be refuted as follows: if learning was affecting subsequent LTP induction by inducing synaptic strength changes, then evidence of these changes should show up as post-training changes in evoked potential size. Such changes were not observed in Experiment 3, where pre-and post-training evoked potentials were directly compared (Fig. 4.5), nor in other studies where such changes have been explicitly sought (*e.g.* Cain *et al.*, 1993). In addition, in Experiment 4 there was no difference in baseline evoked response size between good and poor learners (Fig. 5.14), as might be expected if good learners possessed more training-induced synaptic potentiation. It is thus unlikely that training-

induced synaptic strength changes (if they exist) could be of sufficient magnitude to reduce subsequent LTP induction.

Hippocampal plasticity may mediate non-spatial learning

A second possible explanation is that hippocampal synaptic plasticity is not related to spatial learning. However, if hippocampal synaptic plasticity is really subsuming ability on a non-spatial task, ability on that task would need to be inversely correlated with spatial learning ability in order to explain the present negative correlation between spatial learning and LTP. Such an inverse relationship between two brain competences seems somewhat unlikely, but is not impossible. For example, there is some evidence that rats deprived of their spatial learning ability by hippocampectomy perform better on certain non-spatial tasks (*e.g.* Bunsey and Eichenbaum 1993, Willner *et al.,* 1993), suggesting an interference between the two tasks.

Plasticity upregulation is a compensatory mechanism

A more plausible explanation is that the poor spatial performance seen in Experiment 4 has a cause not related to synaptic efficacy, and that either synaptic plasticity or LTP expression has been upregulated in these animals to compensate. For example, suppose spatial learning ability is related to some factor such as the innervation density of the perforant path, so that rats in which the perforant path is sparse or makes few synaptic contacts with granule cells will perform less well on tasks like the watermaze. Then it may be that NMDA receptor sensitivity or density is increased in these animals to produce partial compensation, the result being an enhancement of LTP induction following tetanisation.

An examination of NMDA receptor function in animals which had participated in Experiment 4 revealed a positive correlation between NMDA current and spatial learning (Fig. 5.36). This correlation did not quite reach statistical significance, but nevertheless there is a clear suggestion that poor learning animals may have smaller intra-tetanic NMDA currents than good learners, implying that LTP may be harder to induce in these animals. In contrast, the negative LTP/learning correlation seen in Experiment 4 was found after LTP had been repeatedly induced over several days: that is, after it had reached "asymptote". As discussed in the previous chapter, animals showing impaired LTP induction (perhaps because of low NMDA receptor density) may show greater LTP expression, in order to keep synaptic efficacy changes within a certain range. If this is the case then the enhanced LTP seen in the poor learning rats in Experiment 4 may have its origins in their smaller NMDA currents, and it follows that NMDA receptor function may be a better correlate of spatial learning than LTP *per se.* Arguing against this explanation is the fact that LTP induction after the first day of tetanisation was not found to be

impaired in the poor learners, as might be expected if NMDA currents were smaller in these animals.

Absent-platform test does not measure only spatial learning

It may be that the measure of "spatial learning" ability used in these experiments: that is, the absent-platform test, does not solely measure this type of learning. Concepts such as spatial learning at present comprise operational definitions and do not yet have a formal description, because they currently lack a complete theoretical basis. In other words, it is not really known what computations are needed for an animal to be able to undertake tasks such as navigating around the environment, and so the term "spatial learning" is loosely applied to tasks which seem intuitively to be spatial. The watermaze task is considered to be a spatial task because it is likely that the other obvious non-spatial methods of problem-solving, such as the formation of simple object-goal associations, could not be used to solve it.

Performance of any task, however, requires the interplay of a large number of ancillary competences: for example, intact sensory functions such as vision and olfaction. It would be generally agreed among experimenters that these faculties, although necessary for solving a spatial task, do not form part of a specific spatial competence. Furthermore, higher cognitive abilities may also be used in the solving of such tasks, even though they themselves do not necessarily comprise "spatial learning" *per se.* In the present study, it may be that what appears to be a correlation with spatial learning is, in reality, a correlation with one of these other functions.

For example, a rat performing a spatial task such as trying to locate a hidden platform in a watermaze needs several simultaneous brain representations: of the environment, of where it is currently situated in that environment and which way it is oriented, of where the goal is, of which is the shortest obstacle-free path to the goal position and so on. If it fails to locate the platform it must then do one of several things: circle round to check its location and orientation within the room, try approaching the putative platform position from a different direction, conduct a more fine-grained search in case it just missed the goal by a small amount or assume that the platform must have moved and try searching somewhere else. Which of these form part of spatial learning and which part of a more general problem solving apparatus is impossible to say given current knowledge. Furthermore, which if any of these is subsumed by the hippocampus (and perhaps its plasticity) is also unknown. Consider a situation in which a trained rat is placed into the watermaze on the absent-platform test and swims to the learned platform position, finding nothing. It may then search persistently in the spot where the platform had been, accumulating a high training quadrant time and earning itself the experimenter-given title of "good learner". A second rat placed into the water under the same conditions, after

swimming to the platform position and finding nothing may then decide to check the rest of the pool to see if the platform had moved: in other words, to attempt to generate a new representation of the goal position. It therefore achieves a low training quadrant time and earns itself, perhaps unfairly, the label of "poor learner". It was found in the present experiment that rats with low training quadrant times showed greater perforant path LTP than rats with high training quadrant times. If that the labels "good learner" and "poor learner" are exchanged for "persistent (or perseverative) searcher" and "flexible searcher", then the association of LTP with performance suggests a new hypothesis: that synaptic plasticity underlies the readiness of a rat to generate a new goal representation under conditions in which the old goal representation failed. On the basis of the current findings, then, it could be tentatively proposed that the function of perforant path plasticity is to detect when a goal representation has failed to match up to reality and to trigger exploratory behaviour in order to update it.

Poor learners show less homosynaptic LTD

A final possibility is that rather than showing enhanced positive synaptic efficacy changes with respect to good learners, the poor learners were deficient in homosynaptic LTD. If tetanisation simultaneously induces both LTP and LTD, the final evoked potential change would represent the sum of both positive and negative synaptic changes. The role of LTD in learning is even less understood than that of LTP, but theoretical models suggest that both types of efficacy change are necessary for optimal information storage (Willshaw and Dayan, 1990). If poor learners were deficient in the LTD component of synaptic change (for example, if the threshold for its induction was higher) the result could be an enhancement of post-tetanisation evoked field potentials with respect to good learners.

6.4 Directions for future research

In many ways, the findings of this thesis raise more questions than they answer. This is a reflection of the complexity of the issues surrounding the mechanisms of learning and memory, and the paucity of our current knowledge. Most of the results found here were unexpected, suggesting that this area is fertile ground for further investigations concerning the contribution of synaptic efficacy changes to learning. Some of the unanswered questions are as follows.

6.4.1 LTP saturation

Does LTP saturation in the hippocampus impair spatial learning?

One question which remains outstanding is whether LTP induction could occlude spatial learning, given the right circumstances. An issue which arises from all of the "saturation"

studies including this one is whether LTP had been induced throughout the whole dorsoventral extent of the hippocampus, in those studies in which an occlusion effect was not seen. In particular, it is possible that the ventral hippocampus received less intense tetanic stimulation than the dorsal, because of the anatomy of the perforant path and location of the stimulating electrodes. Bliss and Richter-Levin (1993) have suggested three methods of testing whether partially or wholly spared ventral LTP may be supporting the learning seen in the rats in these studies. First, if a lesion is placed in the ventral dentate gyrus, then only the DHC should be left to mediate spatial learning. Since LTP is potentially easier to saturate in this region the chances of producing a saturation-related learning impairment would be greatly enhanced. Furthermore, if just enough hippocampus is spared by lesions to support spatial learning (but no more) then redundancy may be so reduced that tetanisation of the remainder becomes sufficient to block learning, irrespective of the location of the lesion within the hippocampus. The latter approach was recently undertaken by Mumby *et al.* (1993), who combined a complete unilateral hippocampal lesion with contralateral tetanisation, producing a consequent impairment of watermaze learning. This finding suggests that if the processing demands on a surviving island of hippocampal tissue are brought sufficiently close to its maximum capability, then tetanisation of that island may be enough to tip the balance and produce a behavioural effect.

The second strategy is to record evoked potentials from several sites along the dorsoventral length of the hippocampus, to investigate whether tetanisation via a different stimulating electrode could subsequently increase LTP at ventral recording sites even when that via the first appeared to have produced "saturation". In this way it would be possible to ascertain whether a single electrode is capable of inducing maximal LTP throughout the whole dentate gyrus. As discussed in Chapter 3, it may be somewhat difficult to produce strong activation of ventral hippocampus using conventional stimulating sites and only a single stimulating electrode. A related approach which circumvents this problem might be to produce generalised synchronous activity throughout the hippocampus by means of seizure induction. The possibility is presently under investigation that electroconvulsive shock (ECS), which appears to impair LTP induction (Anwyl *et al.,* 1987), might do so by inducing LTP throughout the hippocampus and therefore partially saturating it (Stewart *et al.,* 1994). ECS is associated with cognitive impairments in humans, and may result in spatial learning impairments in rats (Stewart and Reid, 1993).

Third, tetanisation of the ventral hippocampal commissure might saturate LTP in the associational-commissural projections to CA1 and CA3. Since the longitudinal association pathway is dense and runs the whole length of the hippocampus (Amaral and Witter, 1989), saturation of this pathway would block further plastic changes throughout

the hippocampus. An alternative approach could be to find a pharmacological means of saturating LTP. NMDA application only produces STP (Kauer *et al.,* 1988) which would not last long enough to be of practical use. At present it has not been possible to produce pharmacologically induced LTP, although it is possible that some combination of NMDA and metabotropic receptor activation might be successful. If LTP can be induced by drug application without producing concomitant seizure activity then this technique would provide a useful means of testing the occlusion hypothesis.

An LTP-induced occlusion of spatial learning is a strong prediction of the plasticity/learning hypothesis and so confirmation of the effect would therefore greatly support it. A persistent failure to find the effect would be less easy to interpret, for the reasons mentioned in Chapter 3: that is, there are many theoretical reasons why it might not be practical to induce LTP sufficiently strongly in enough synapses to impair learning, even if synaptic strength changes *are* involved in the learning process. However, the finding of Mumby *et al.* (1993) that a tetanisation-related impairment of watermaze learning can be produced in a partially lesioned hippocampus suggests that the occlusion effect may yet be reproducible under certain conditions.

6.4.2 Correlation of LTP with spatial learning

What is the mechanism of LTP expression?

This question arises because of the finding of the current study that *cumulative* LTP magnitude correlates with spatial learning: that is, only after several episodes of repeated tetanisation does a separation emerge between the potentiation expressed by good and poor learners (Fig. 5.22). Repeated tetanisation appears to produce asymptotic LTP, suggesting that each animal possesses an intrinsic maximal synaptic efficacy beyond which the synapses cannot be driven, given constant stimulus conditions. This maximum has been referred to here as the LTP "ceiling". The factors governing the magnitude of this ceiling are currently unknown but the finding that it correlates well with spatial learning suggests that it may be related to information storage, and so an elucidation of these factors is indicated. The slow time course of the development of the LTP/learning correlation suggests that induction and expression are dissociable, and raises the possibility that enhanced LTP magnitude may be a compensation for reduced synaptic plasticity. A resolution of these issues is of some importance as it would enable the formulation of a computational theory of memory formation which assigns separable roles to these two aspects of LTP.

Is LTP increased or decreased in poor learning rats?

The most surprising result to emerge from the current study was that LTP was greater in poor learning rats when evoked potentials were sampled using a low-strength stimulus current. This finding prompted an exploration of the relationship between LTP and stimulus intensity, whereupon it was discovered that LTP declined exponentially with increasing stimulus strength at a rate proportional to the log of its magnitude. The hypothesis was then advanced that the decline of LTP across the IO curve was due to interactions between EPSPs, and that larger (*e.g.* potentiated) EPSPs interacted more than smaller ones. Thus, the LTP measured using small stimuli is arguably a more appropriate measure because the interactions are less at low current strengths, and by extrapolation the best measure would be the LTP measured at a near-zero current, where the interactions would be negligible. When this measure was derived by extrapolation and used to represent the LTP value for a given rat, the correlation with learning was strongly negative. The conclusion is that LTP expression is greater in poor learning animals.

This conclusion is a rather important one and so the question of whether the above argument concerning LTP estimation is correct needs to be resolved. One possible approach would be by means of intracellular recording in the hippocampal slice. Voltage clamping eliminates influences on the evoked response of such factors as changes in driving force and membrane conductance and would enable determination of the unitary excitatory postsynaptic currents (EPSCs). If LTP involves a change in this parameter then it should be possible to estimate the "real" percentage potentiation for a given animal. If the preceding hypothesis is true, the estimate of unitary EPSC potentiation should be comparable with that obtained by the backwards-extrapolated linearised LTP/current curve.

Role of the perforant path in spatial learning

The finding of a negative correlation between LTP and performance on the watermaze task is puzzling. One possible explanation is that the absent-platform test, which was used throughout this study to measure spatial learning ability, is not measuring information storage but some other competence. Since it is not known how the spatial learning function of the hippocampus can be decomposed, the possible role of the perforant path in solving tasks like the watermaze can only be hypothesised at present. One interesting possibility, discussed earlier, is that it is associated with searching behaviour, and that the role of synaptic efficacy changes in the perforant path is in the generation of a new representation of the goal location. If this is the case, then interrupting perforant path plasticity without affecting any other hippocampal functions might be expected to produce perseveration (inflexibility of search) when a rat is faced

with a task like the absent-platform test. This hypothesis is difficult to test at present because it is not possible to infuse NMDA antagonists into a sufficiently localised area, and perforant path transection affects other parts of the hippocampal circuitry as well as damaging the entorhinal cortex (Skelton and McNamara, 1992). However, if the LTP saturation technique becomes validated then it would be interesting to observe the effects of saturating perforant path LTP on search behaviour. In this context, it is worth noting that the first saturation study employed a reversal task in which "saturated" rats persisted in searching in the former goal location and failed to learn the new one.

6.5 Conclusions

The experiments described in this thesis set out to explore the possibility that hippocampal synaptic efficacy changes are related to the spatial learning function of this structure. Two main results emerge from the 5 experiments presented here. The first is that contrary to both theoretical predictions and previously reported findings, maximal induction of LTP in the perforant path does not appear to impair the learning of a spatial task which is known to depend on the integrity of this pathway. The second is that nevertheless, cumulative perforant path LTP correlates with performance on the task, supporting the hypothesis that there may be a link between synaptic efficacy changes and learning.

Paradoxically, however, it appears that this correlation may be negative, a conclusion which derived from the observation of an inverse relationship between the amount of LTP and test current strength, such that animals showing the greatest potentiation at low stimulus intensities showed the least at high intensities. It was argued here that low stimulus intensities may provide a more accurate measure of synaptic efficacy, in which case the most accomplished spatial learners demonstrated the least amount of LTP. This result raises important questions about the possible contribution of perforant path synaptic efficacy changes to learning.

The finding of a correlation between perforant path LTP and spatial learning ability does not, by itself, prove that LTP is causally related to learning. It does, however, add circumstantial evidence to support the hypothesis. The finding that this correlation may be negative at the level of potentiation of individual synapses is contrary to the simplest predictions of most computational theories of learning, suggesting that if LTP really is contributing to learning, the relationship may be less straightforward than might be expected. Some possible reasons for this apparently paradoxical finding have been presented above. The question of the relationship between LTP and learning will probably not be fully resolved until spontaneous synaptic efficacy changes can be observed in a naturalistic learning situation, and shown to depend on the same cellular

machinery as does LTP itself. However, the findings presented in this thesis add to the growing body of evidence supporting the role of an LTP-like process in the hippocampus in subsuming spatial learning. In addition, they suggest a re-examination of the way in which synaptic efficacy changes are measured in the behaving animal.

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Appendix

A description is presented below of several other studies investigating the effects of maximal induction of LTP on spatial learning. Most of the studies were conducted virtually simultaneously and presented together, along with Experiments 1 and 2 of this thesis (Jeffery and Morris, 1993) in *Hippocampus,* vol. 3(2).

Robinson, (1992) *Hippocampus,* 2(4): 389-396. This experiment was designed to explore the possibility that maximal LTP induction in the perforant path might impair the acquisition of reference memory in the radial arm maze, even though it did not appear to affect working memory in the same apparatus (McNaughton *et al.,* 1986). LTP was therefore induced over 5 days before any behavioural training began, and during the course of training tetanic stimulation continued to be administered daily, "immediately" prior to each day's training trials. Thus the experiment examined the possible anterograde effects of tetanic stimulation of the perforant path on subsequent spatial learning. After rats had learned the standard 8-arm maze task they began training on the 4/8 version of the task, and were scored according to reference memory errors (entering arms other than the 4 baited arms) and working memory errors (re-entering arms after food had already been consumed there). There was no difference in performance between tetanised rats and low frequency controls. A correlation was found between LTP and spatial learning although this varied inversely for the EPSP and population spike, and was not statistically significant.

Korol, Abel, Church, Barnes and McNaughton, (1993) *Hippocampus,* 3(2): 127-132. This study was conducted by the group responsible for the original saturation studies (McNaughton *et al.,* 1986, Castro *et al.,* 1989) and was an attempt to replicate the watermaze study. In this paper they reveal that the Castro *et al.* study had used rats previously housed in an enriched environment, as part of another experiment. The possibility was therefore considered that changes in hippocampal physiology brought about by enrichment (for example, partial saturation of synaptic weights by enrichment-associated naturally occurring LTP) might have produced greater sensitivity to the effects of LTP saturation. Rats were therefore subjected to experience with an enriched environment (complex housing) before undergoing a 12-day course of daily LTP induction and then watermaze training. No deficit in spatial learning was seen.

Sutherland, Dringenberg and Hoesing, (1993) *Hippocampus,* 3(2): 141-147. In the first of two experiments, rats undergoing a course of LTP saturation according to the Castro *et al.* protocol were trained in 2-trial blocks on a working memory version of the

watermaze task, in which a rat is required to learn the current position of the platform on trial 1 and recall that position on trial 2. The platform was shifted between blocks. Even when training blocks were interspersed with tetanisation sessions over the 14-day period, the rats learned normally. When trained in a different watermaze in a new room over the two days following tetanisation they still performed as well as controls. In the second experiment the procedure of Castro *et al.* was followed exactly, with the exception that training began 24 h after the last tetanisation, rather than 5-10 min. These rats also learned normally.

McNamara, Kirkby, dePape, Skelton and Corcoran, (1993) *Hippocampus,* 3(2): 149-152. In this experiment, place learning in the watermaze was impaired if the rats had received a kindled seizure shortly prior to training, but kindling alone was not sufficient to affect performance. This finding rules out effects on behaviour such as stimulation-related perforant path damage, and suggests that widespread induction of synaptic efficacy changes such as those occurring after kindling are not sufficient to impair learning.

Cain, Hargreaves, Boon and Dennison, (1993) *Hippocampus,* 3(2): 153-163. These authors conducted a two-part study looking at (a) the effects of watermaze training on evoked potentials, and (b) the effects of bilateral "saturation" of LTP on watermaze training, following the Castro *et al.* protocol. LTP was induced using pulses near or well above the intensity needed to induce maximal responses as measured on the IO curve, to increase the chance of activating all perforant path fibres and therefore saturating LTP. The second part also included a comparison of two other types of hippocampal stimulation: application of 60 Hz stimulation to the perforant path unilaterally until a single afterdischarge was produced, or daily application of the afterdischarge-producing stimulation (kindling) bilaterally until stage 5 seizures were produced in one hemisphere. Evoked responses were measured using low, medium and high intensity pulses.

Prior watermaze training produced good learning and retention for at least 24 h, but no apparent change in the evoked responses measured either 1 h or 24 h later. LTP was subsequently induced successfully, demonstrating that the procedure was capable of detecting plastic changes if they were sufficiently large. Bilateral LTP induction to asymptotic levels did not affect watermaze performance even when training was begun only 5-10 min after the last tetanisation session, while both the afterdischarge group and the kindled group were impaired when training was administered at a similarly short interval following the final stimulation. A second absent-platform test performed 24 h later, however, revealed performance well above chance in the latter two groups, suggesting that some learning had taken place. Further learning proceeded normally on the second day, suggesting that the previous day's learning impairment was likely related

to transient disruption resulting from the seizure activity, and not to longer lasting changes in synaptic plasticity.

One final finding was that LTP declined greatly with increasing stimulus intensity, being 130%, 40% and 13% for the population spike evoked at low, medium and high intensities respectively. Furthermore, some of the evoked response measures correlated with learning, two negatively and one positively, though few details were given and the correlation was attributed to chance by the authors. The possible significance of both the variation in measured LTP and the negative correlations is discussed in Chapter 5.

Mumby, Weisend, Barela and Sutherland, (1993) ***Society for Neuroscience Abstracts,* 19: 186.2.** Rats were given unilateral neurotoxic hippocampal lesions, and then received 20 consecutive days of contralateral daily tetanisation. Watermaze training took place 15 min after each of the last 7 tetanisation sessions. Rats which had received tetanisation were significantly impaired on the absent-platform test compared with low-frequency-stimulated controls.